

Figure 1: Examples of Nuclease Stable Ribozyme Motifs

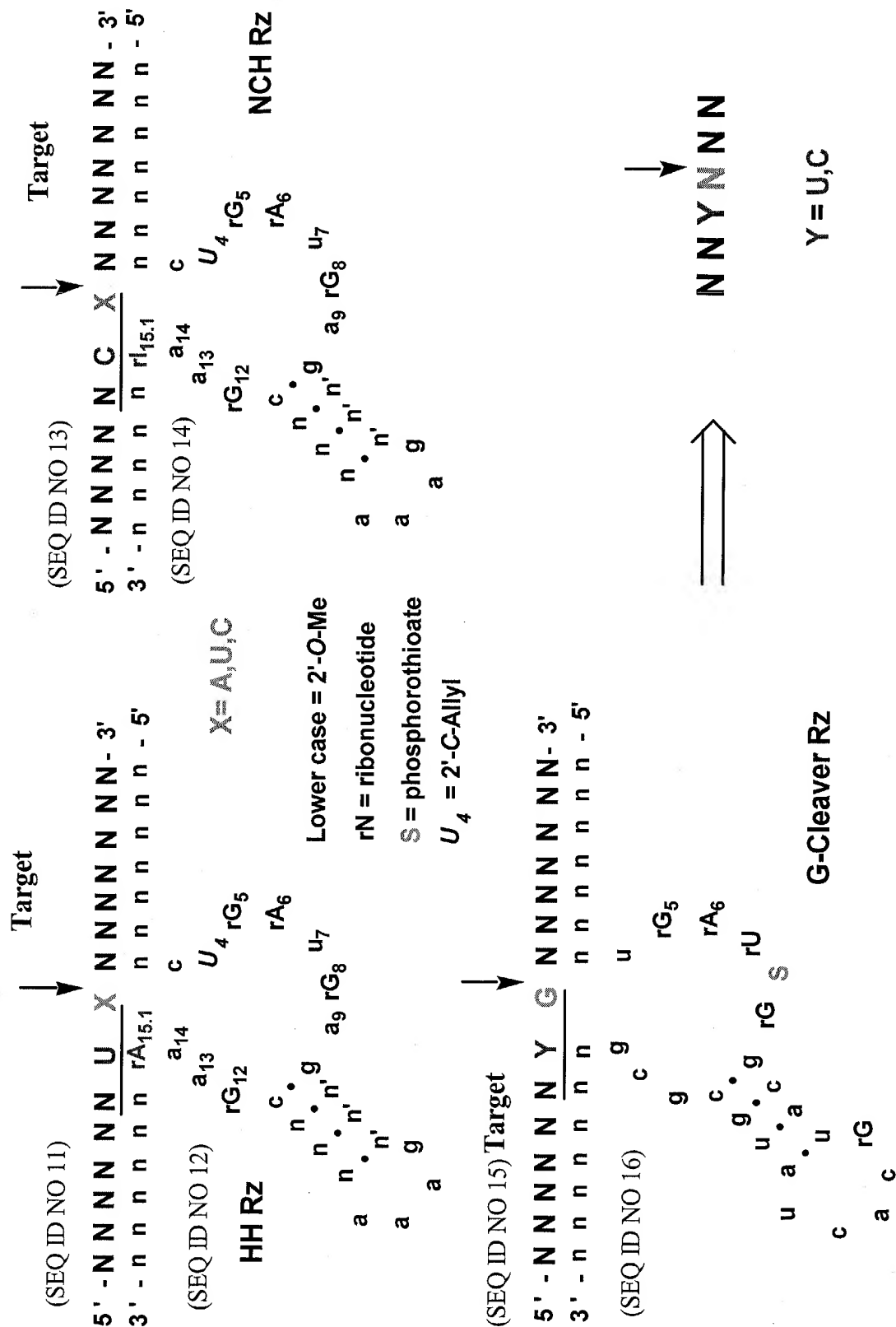


Figure 2: 2'-O-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

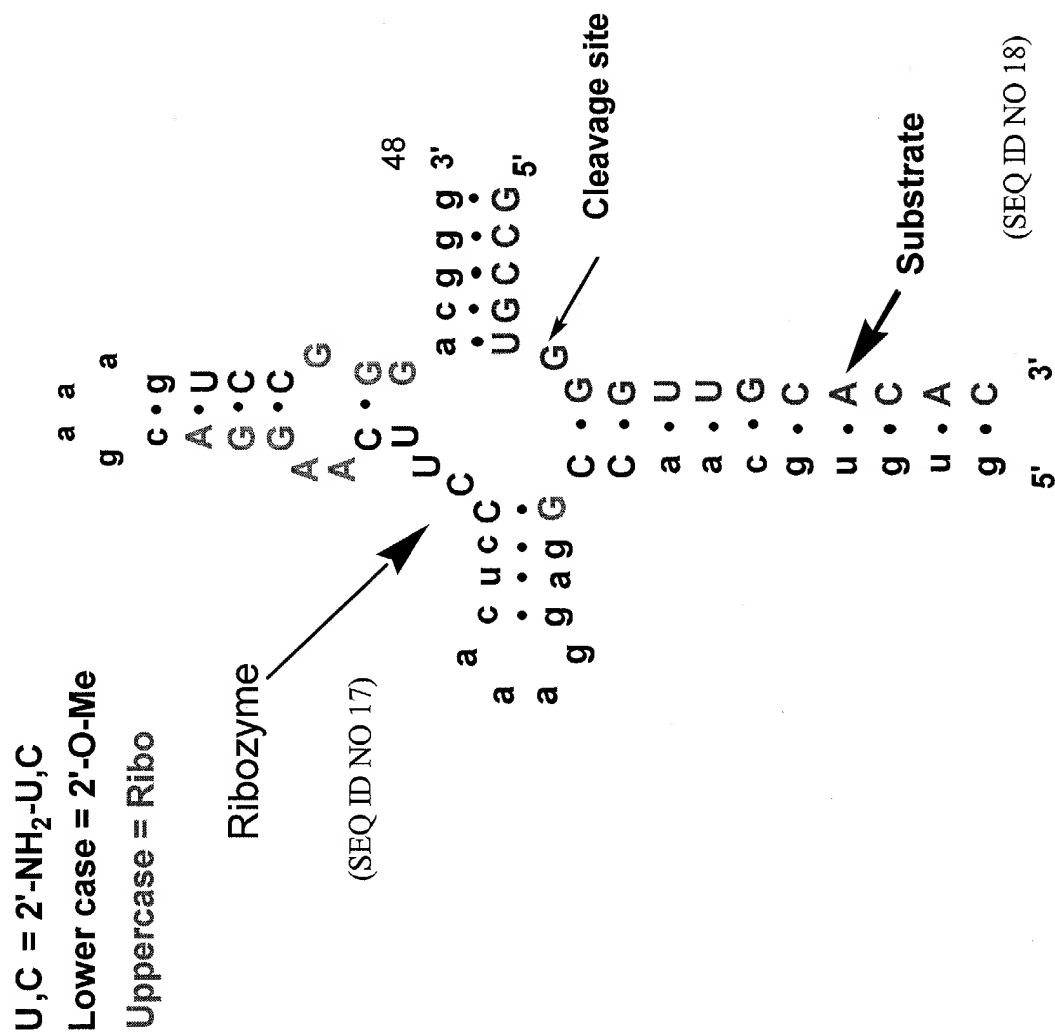
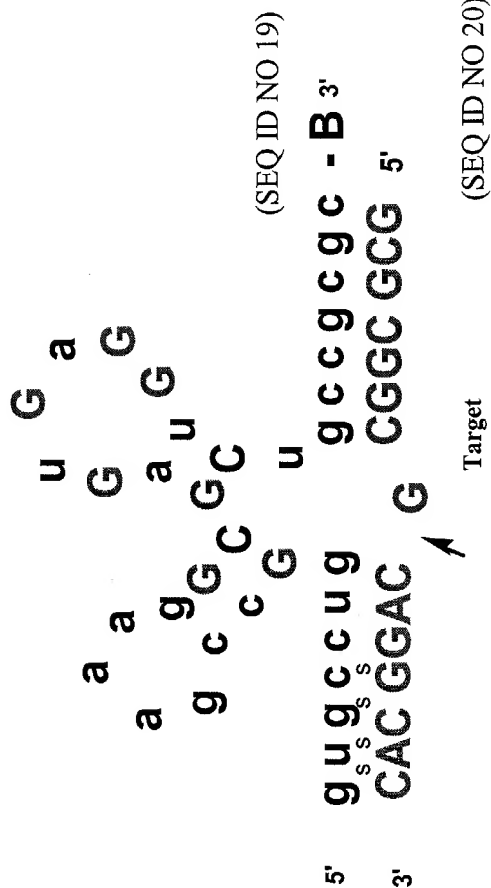


Figure 3: Stabilized Zinzyme Ribozyme Motif

## Zinzyme A-motif RZ



### Legend

Uppercase indicates natural ribo residues

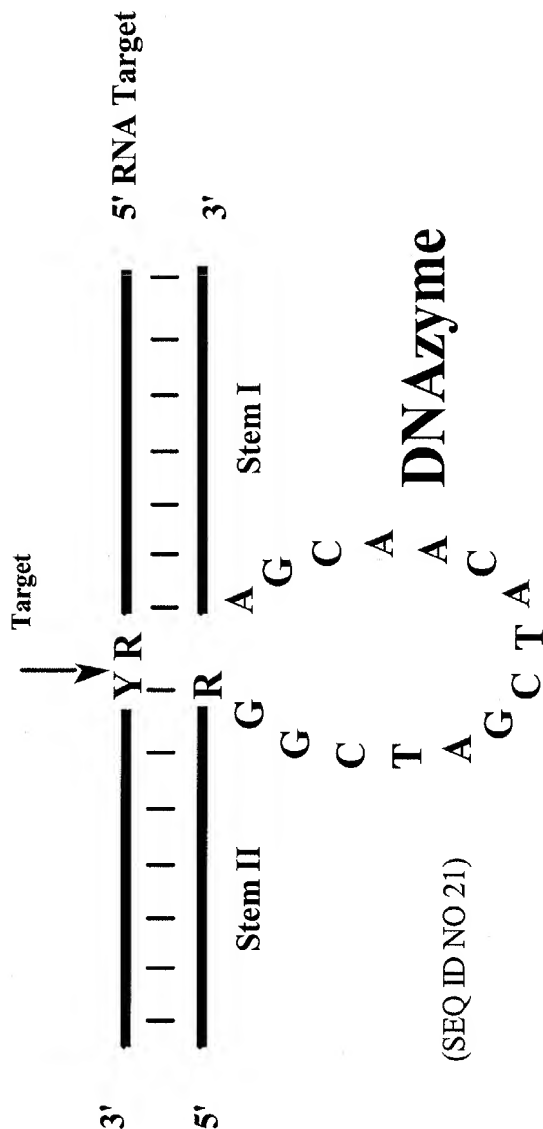
**C** indicates 2' - d-NH<sub>2</sub>-C

Lowercase: 2'-O- Me

Subscript <sub>s</sub> indicates phosphothioate linkage

B: 3'-3' abasic moiety

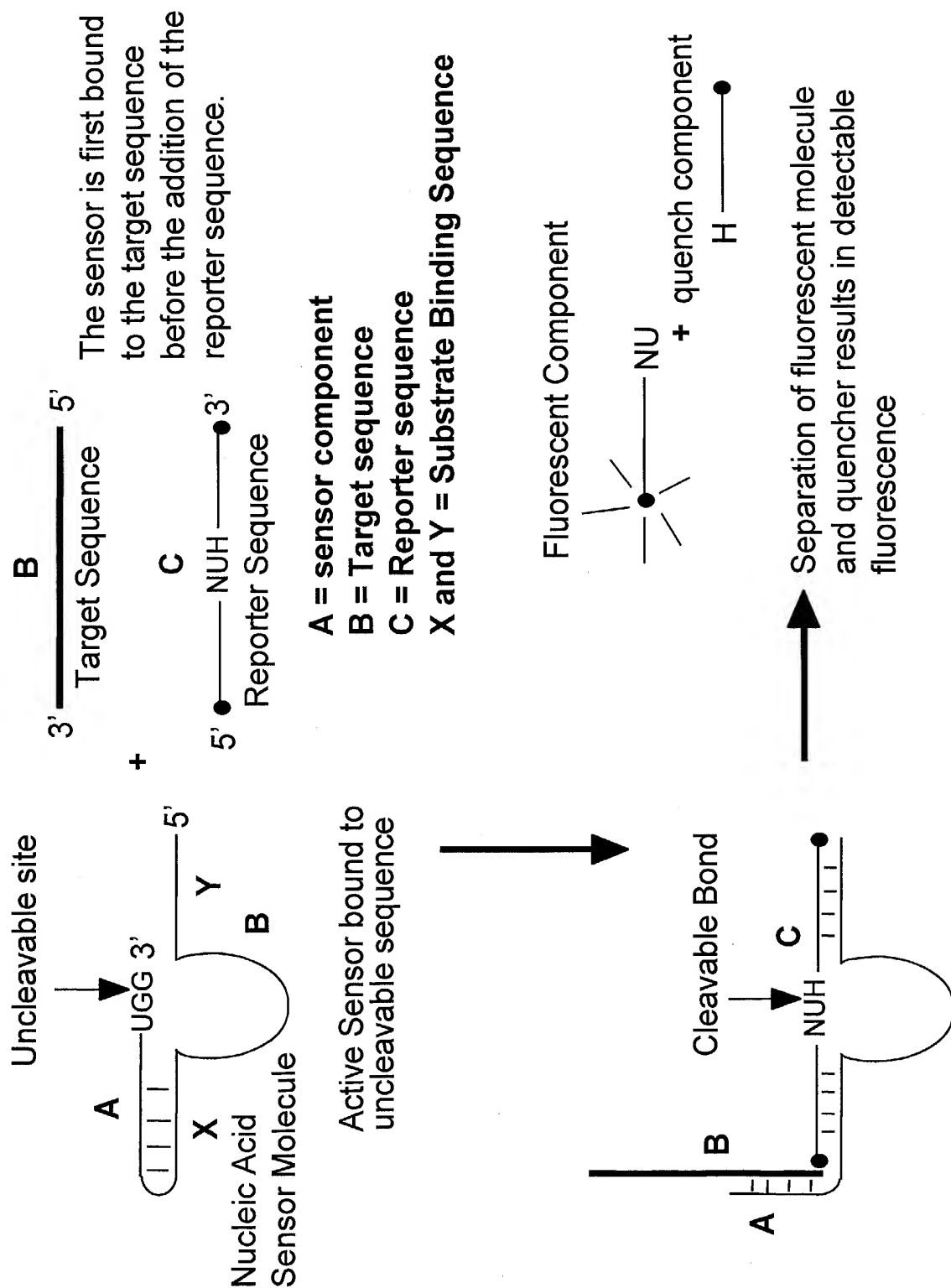
Figure 4: DNAzyme Motif



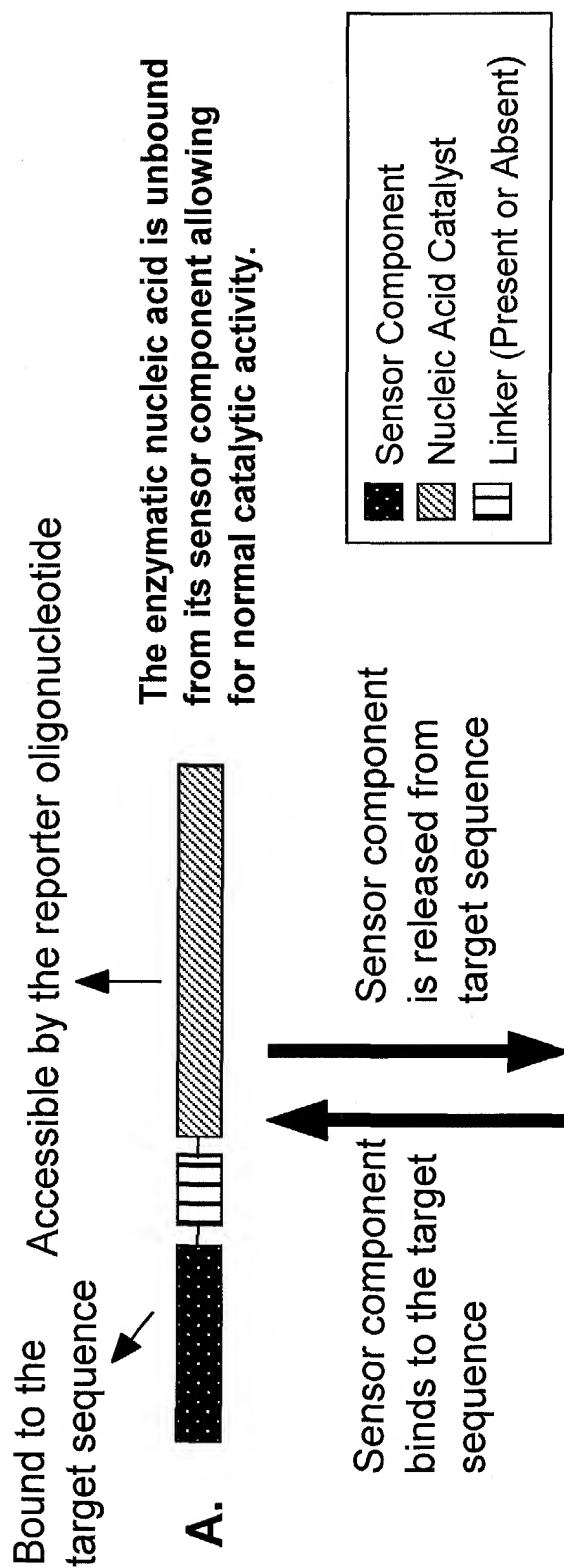
Legend

Y = U or C  
R = A or G

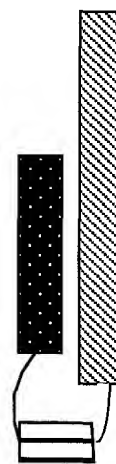
Figure 5. Detection of Target Sequence Using a *Cis-Blocking Sequence*



**Figure 6. Schematic Diagram Representing the Two Primary Configurations of the Diagnostic effector molecule**

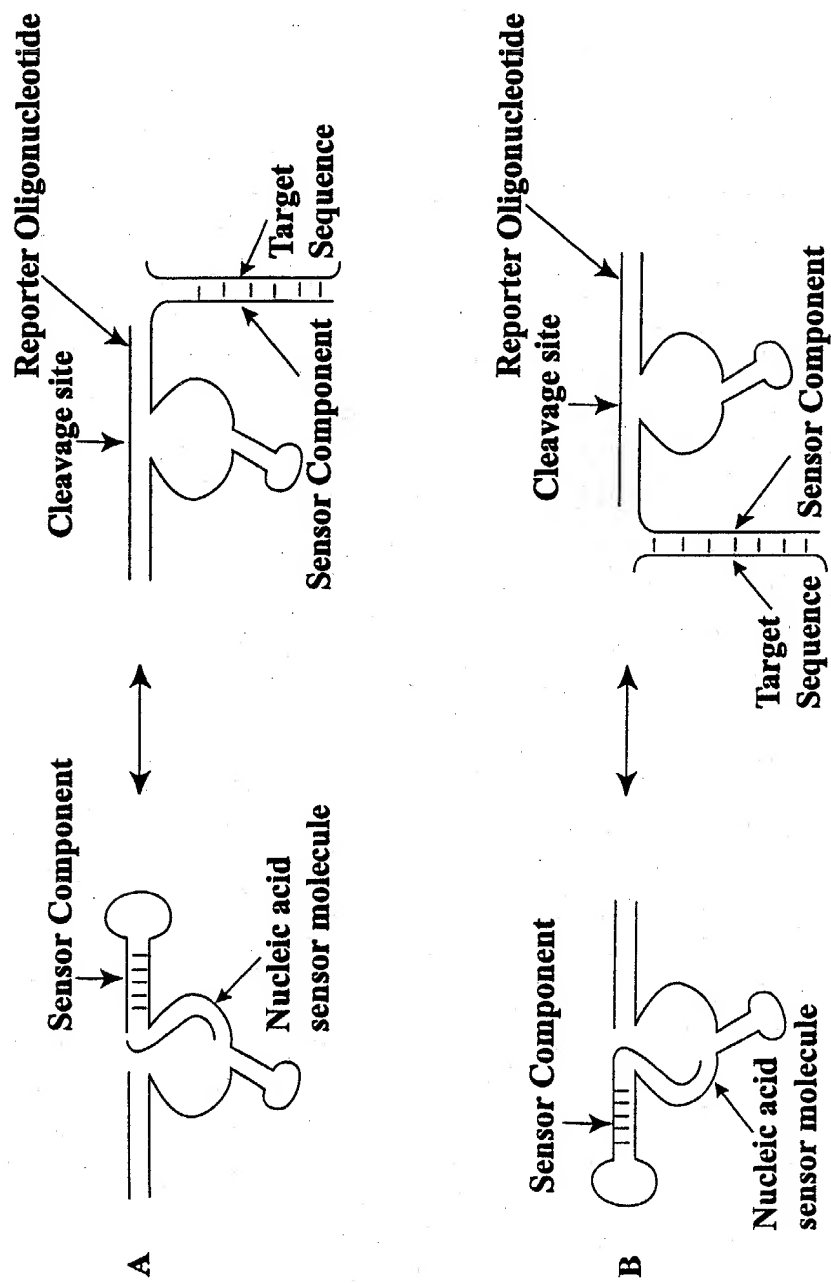


The nucleic acid catalyst binds to the sensor component which prevents the catalytic activity of the molecule. Binding is to either the substrate binding arms or to nucleotides within the sensor molecule core.

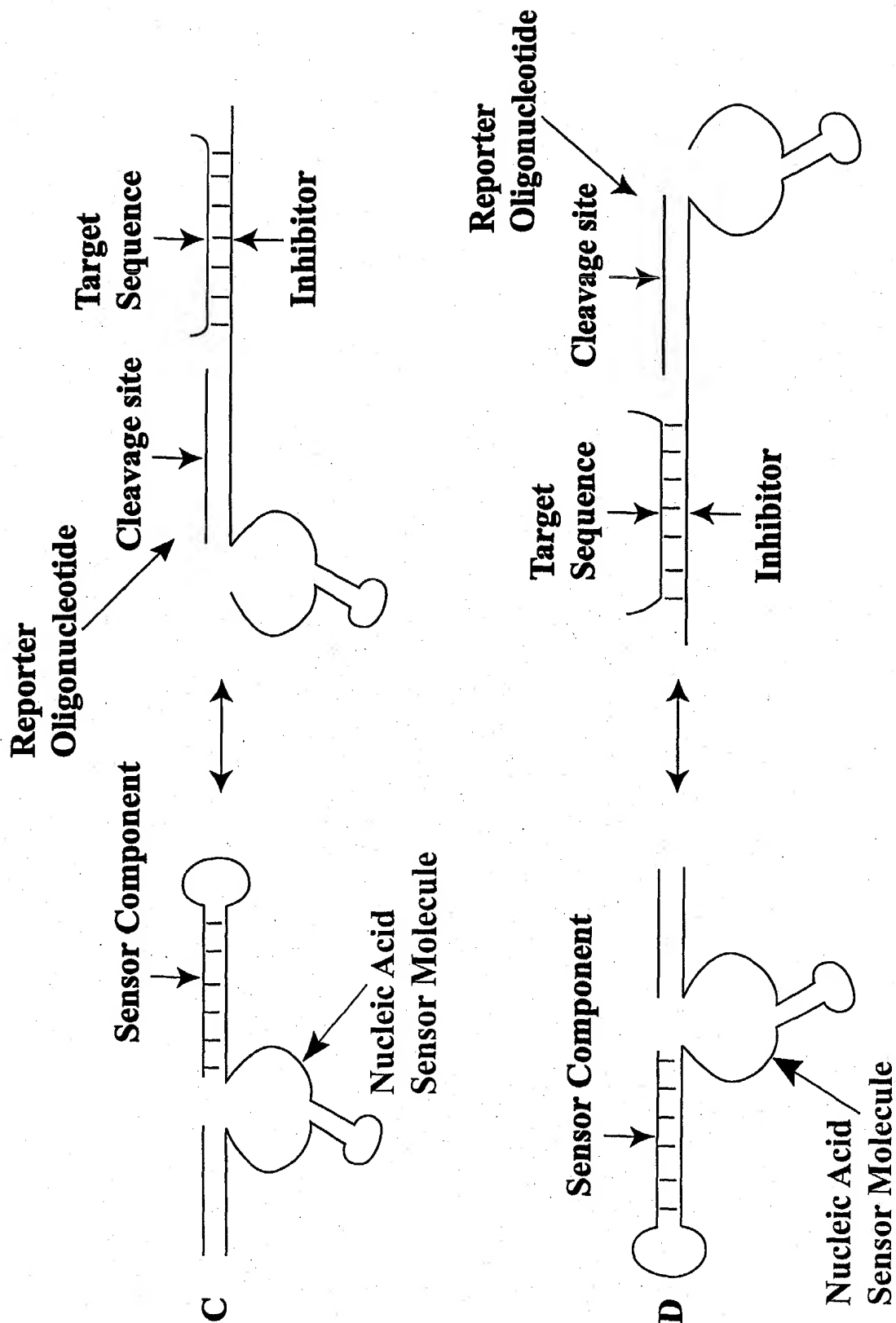


The catalyst region is now inaccessible to the reporter sequence

**Figure 7a. Examples of Diagnostic Effector Molecules**



**Figure 7b. Examples of Diagnostic Effector Molecules**





**Figure 8a. Examples of Diagnostic Effector Molecules**

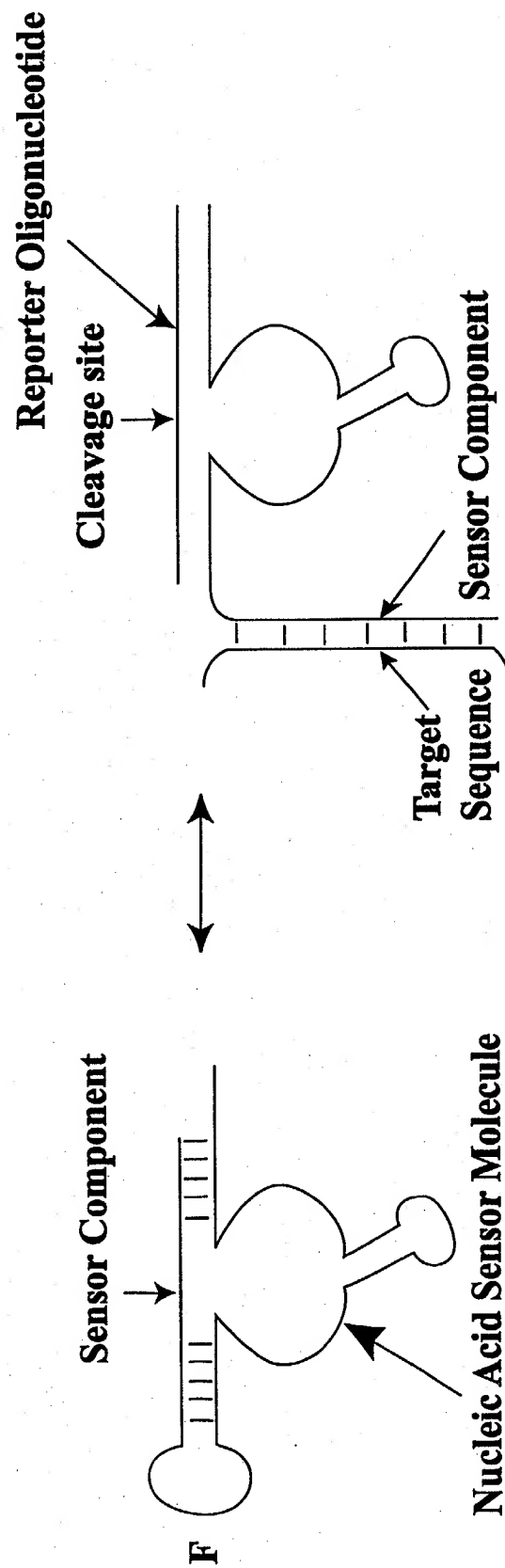
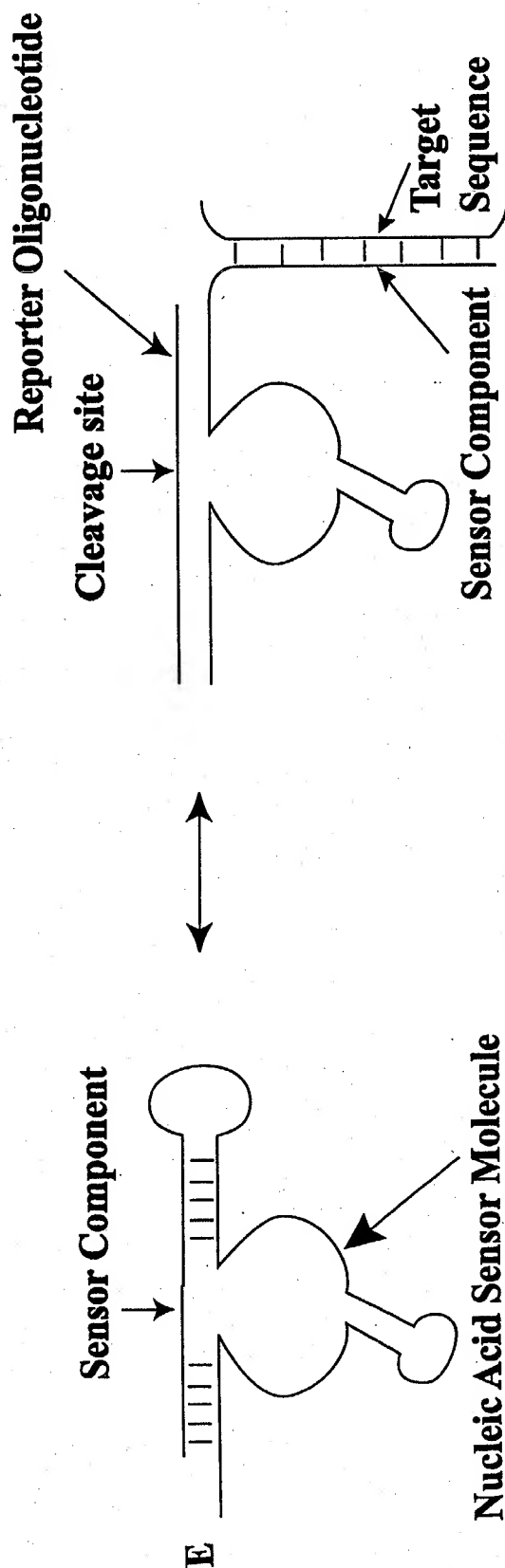
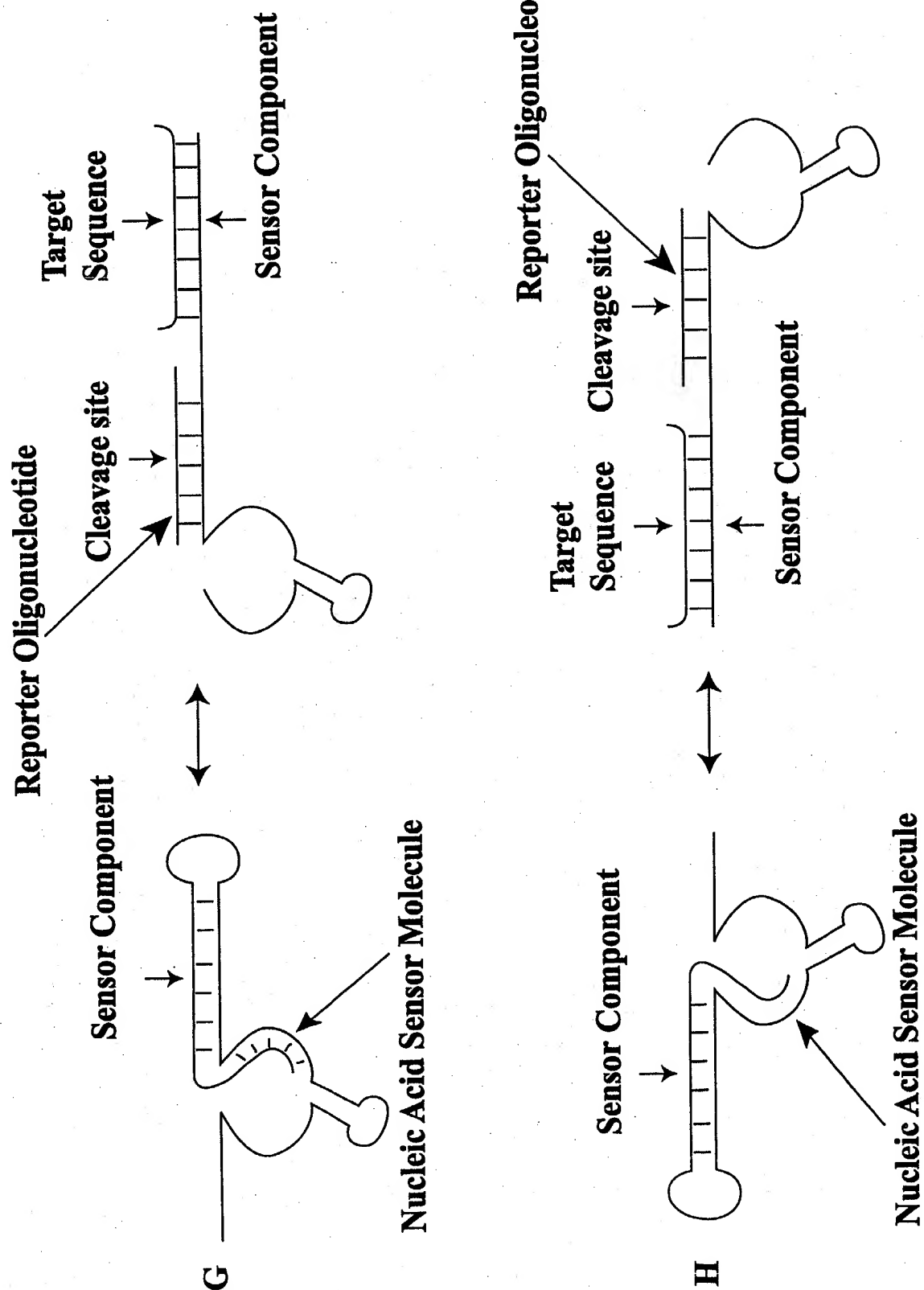
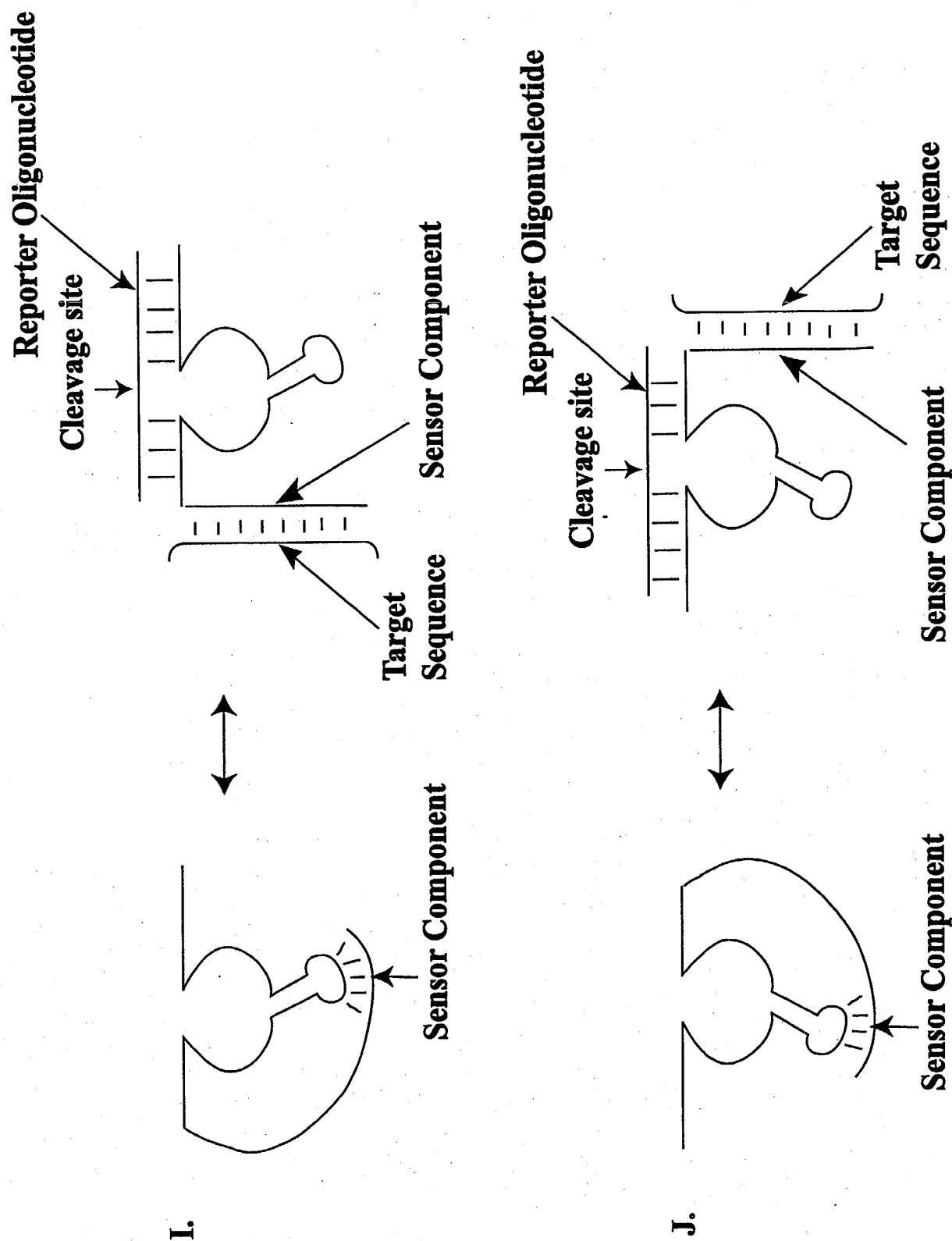


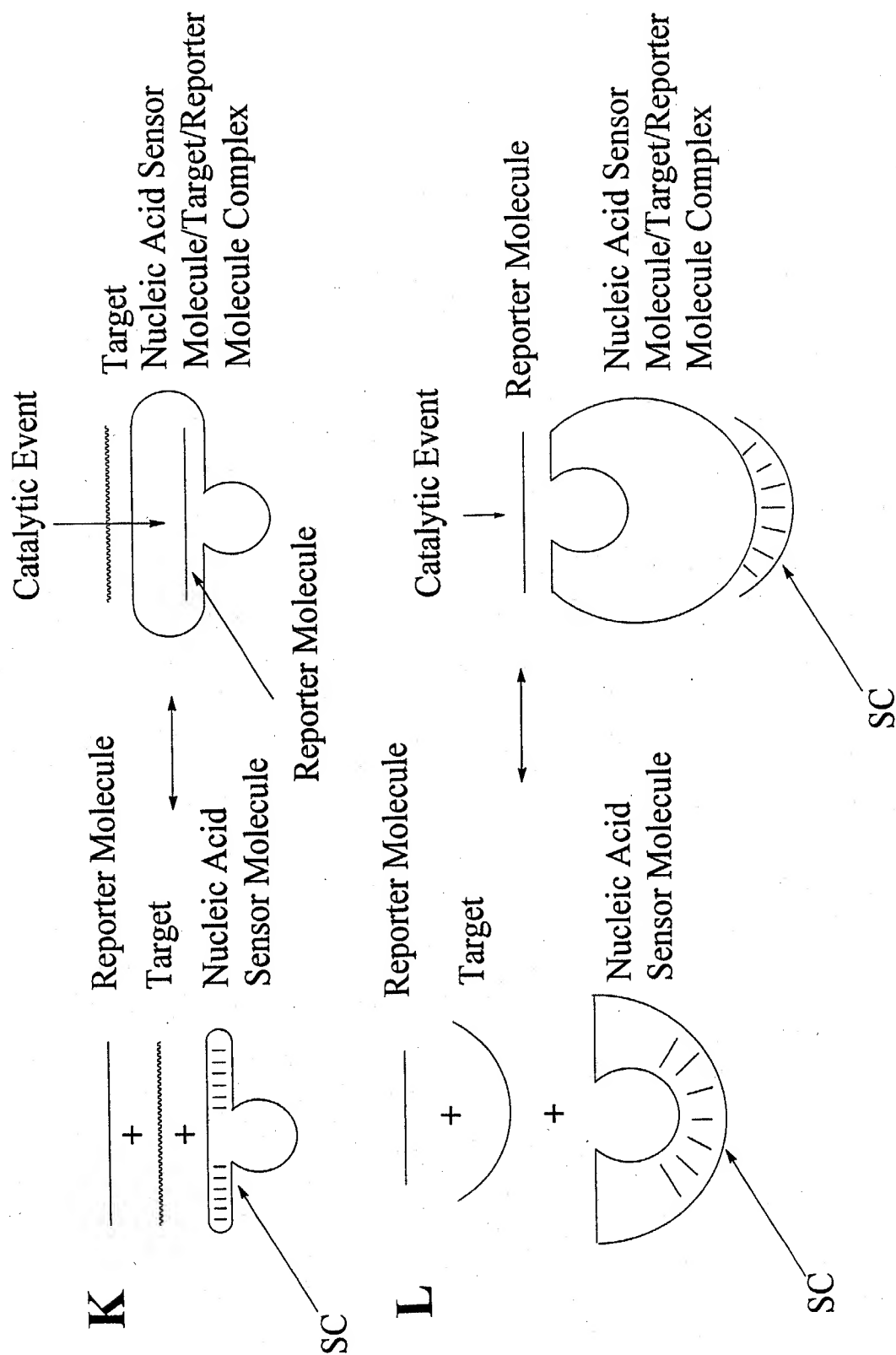
Figure 8b. Examples of Diagnostic Effector Molecules



**Figure 9. Examples of Diagnostic Effector Molecules**



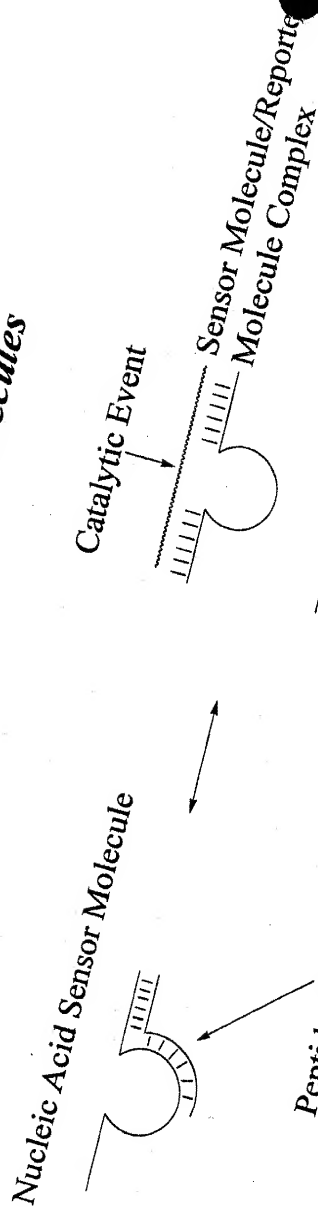
**Figure 10: Examples of Diagnostic Effector Molecules**



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**Figure 11: Examples of Diagnostic Effector Molecules**

**M**



**N**

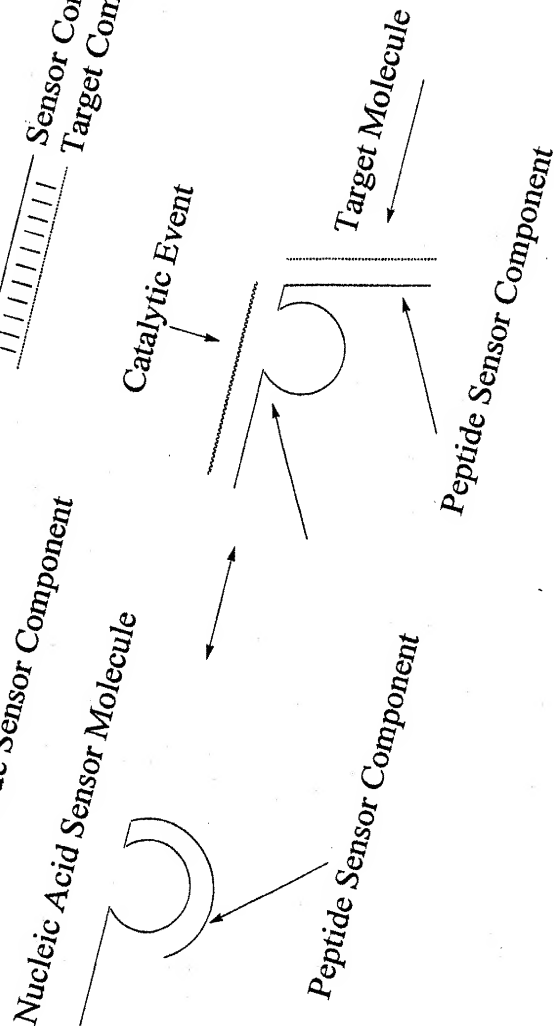
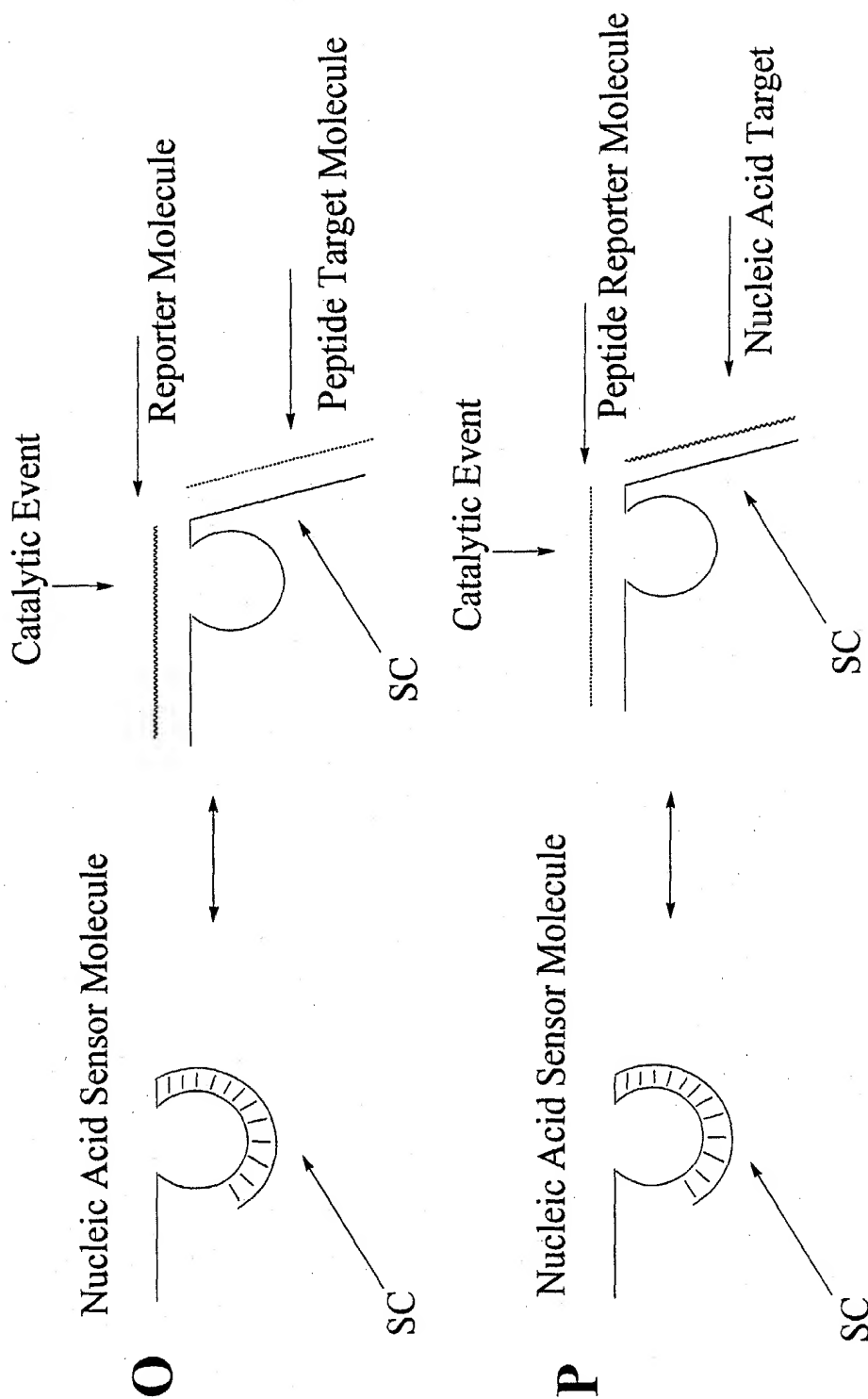


Figure 12: Examples of Diagnostic Effector Molecules



SC = Sensor Component

Figure 13: Examples of Diagnostic Effector Molecules

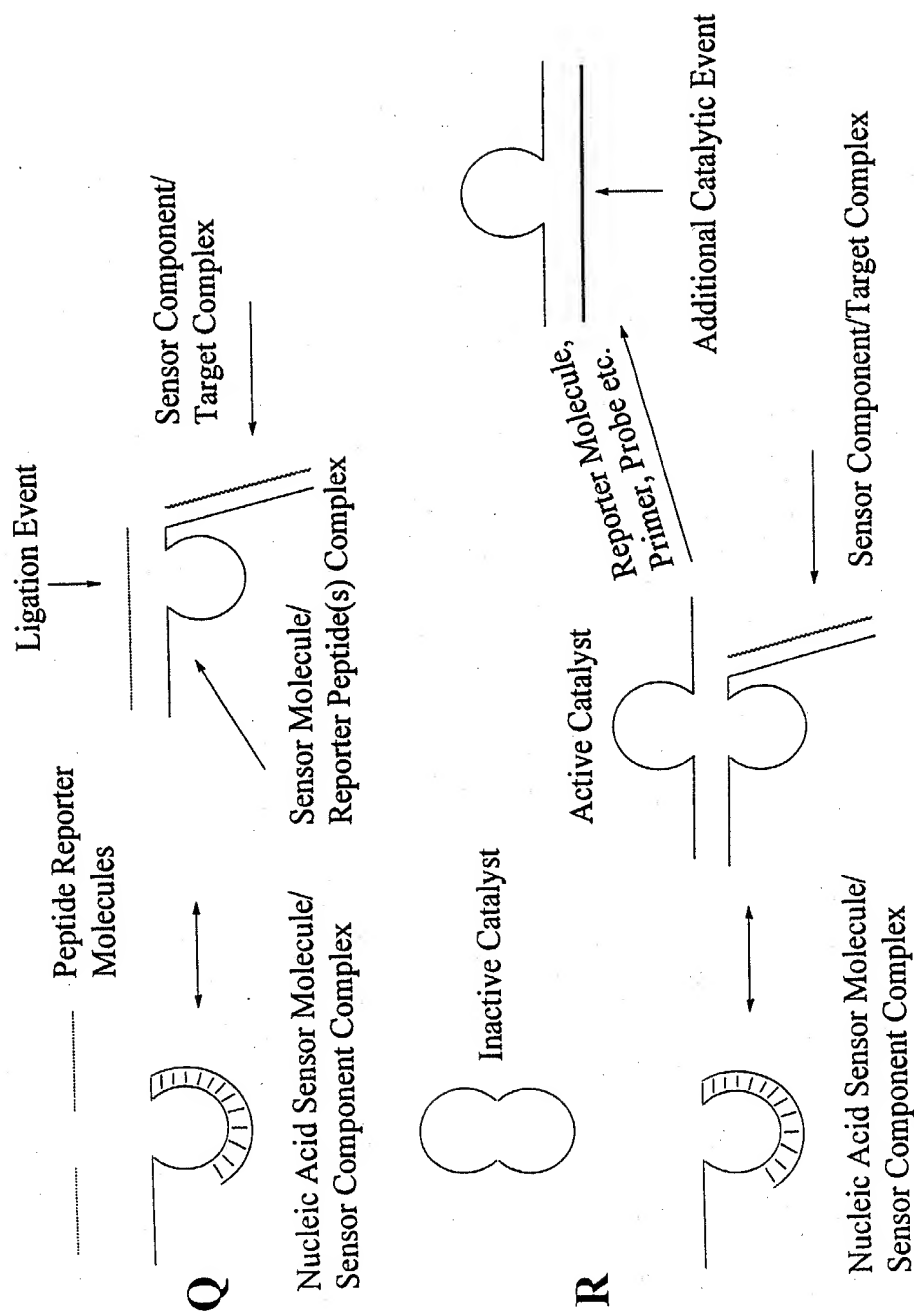


Figure 14: Inherent Amplification of Signal

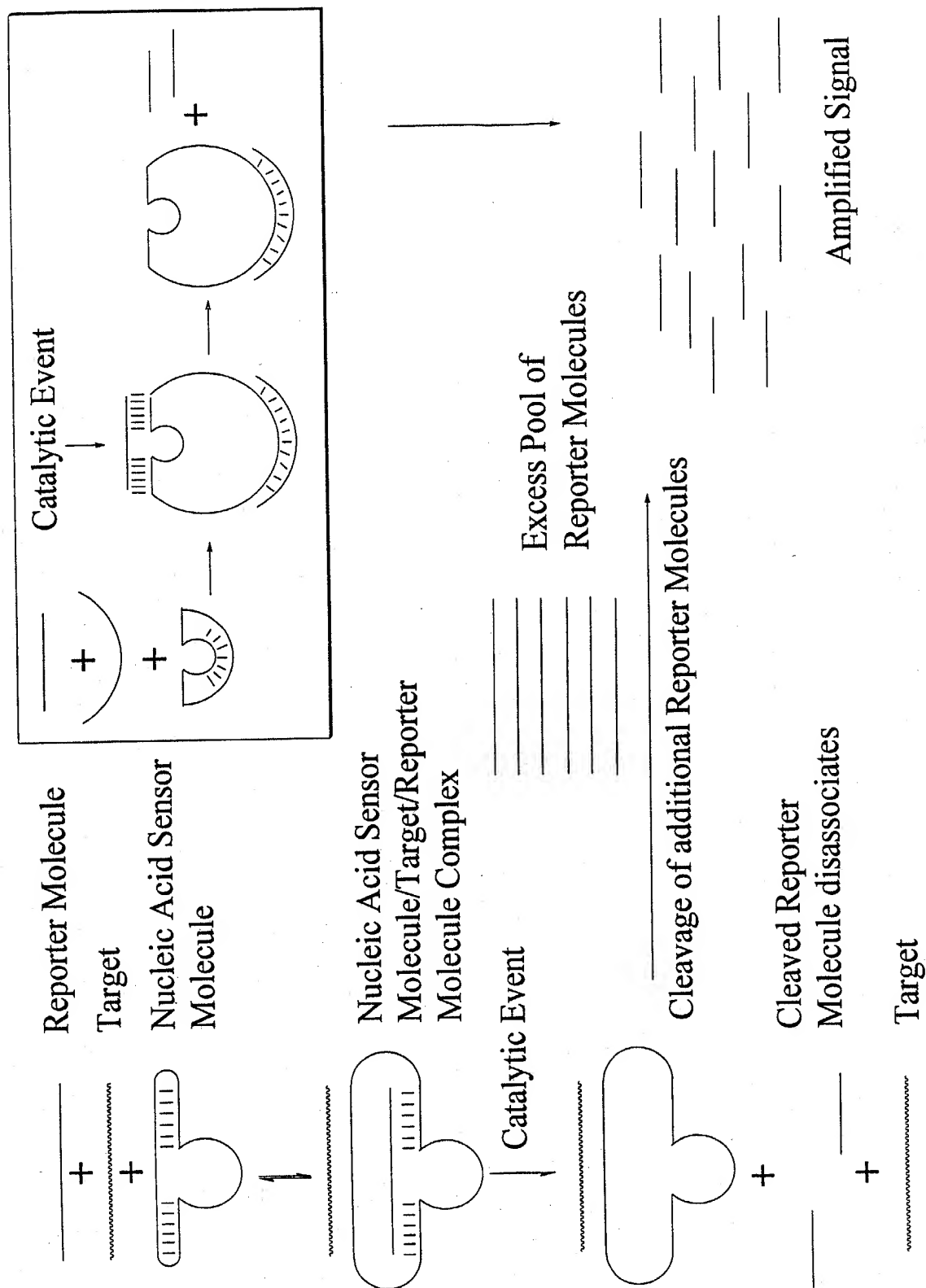
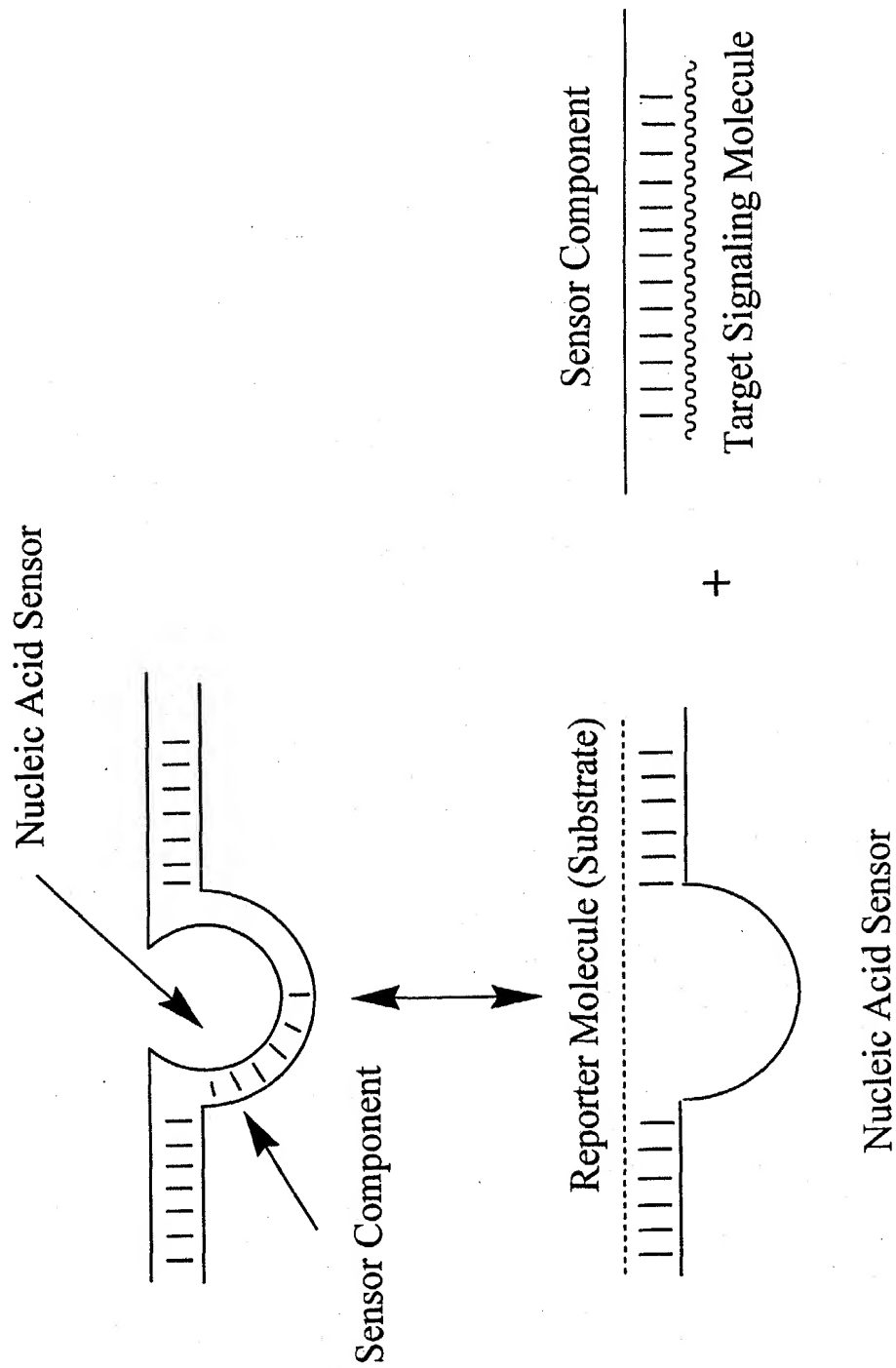




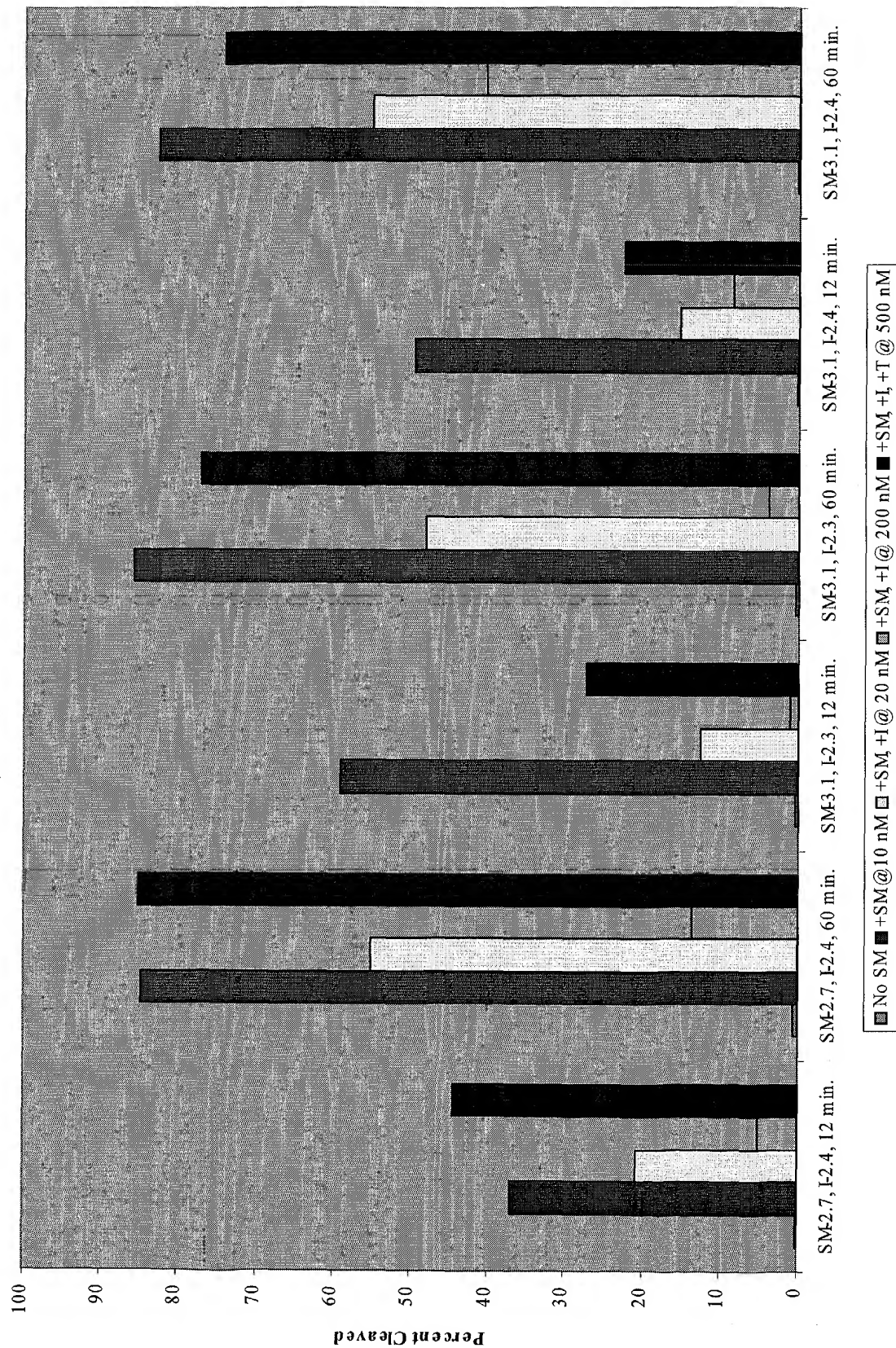
Figure 15: Example of Diagnostic System



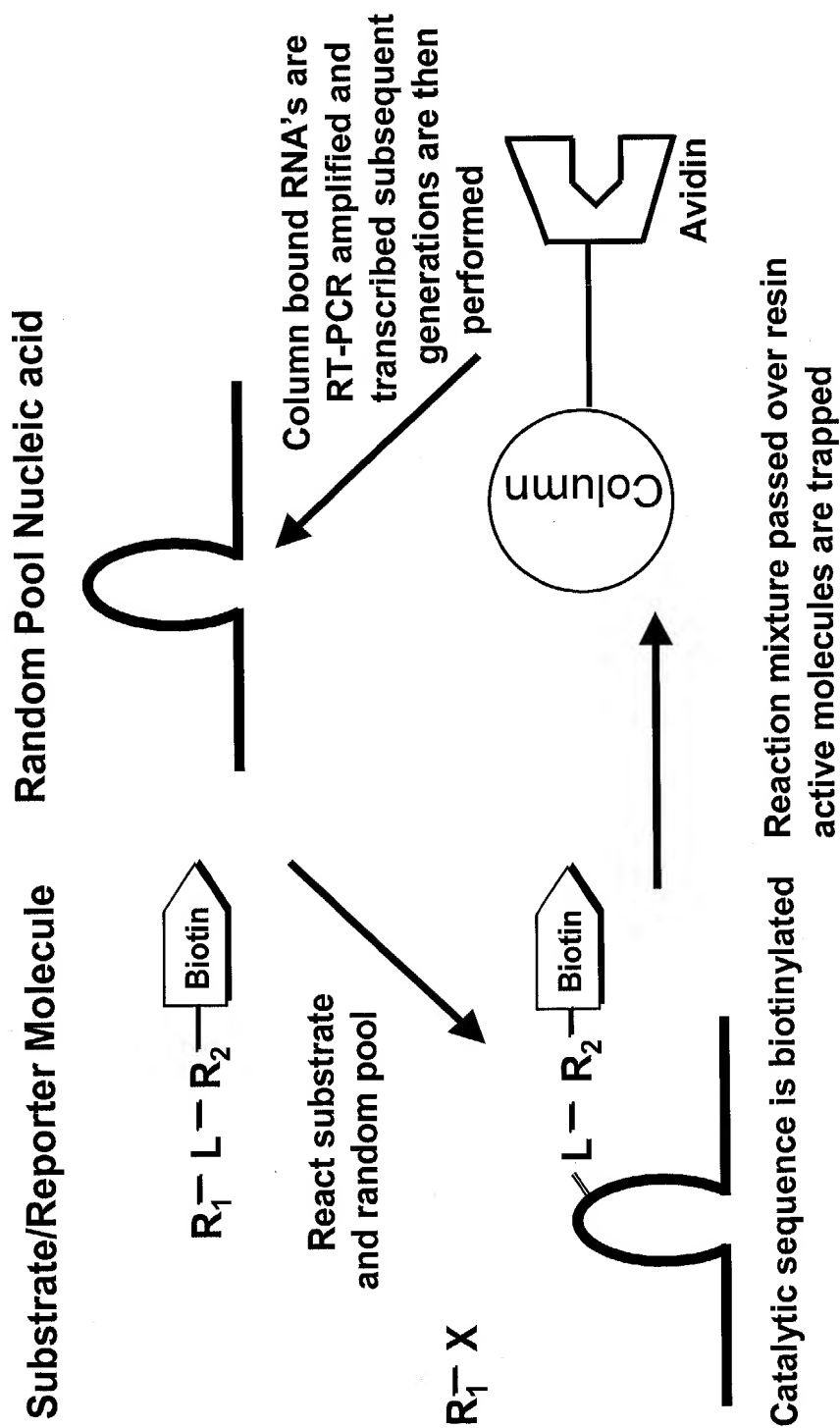
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Figure 16: Diagnostic Screen

Inhibitory Folding with Target Rescue



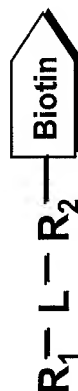
**Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme**



# Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules - Ligand Dependent

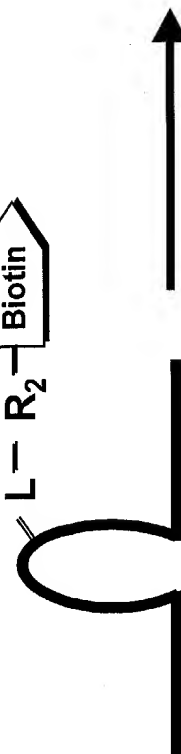
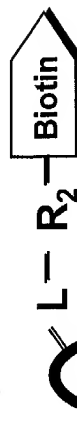
Substrate/Reporter Molecule + Random Pool Nucleic acid

- Ligand (first round)
- + Ligand (second round)



React substrate  
and random pool

$R_1 - X$



Catalytic sequence is biotinylated

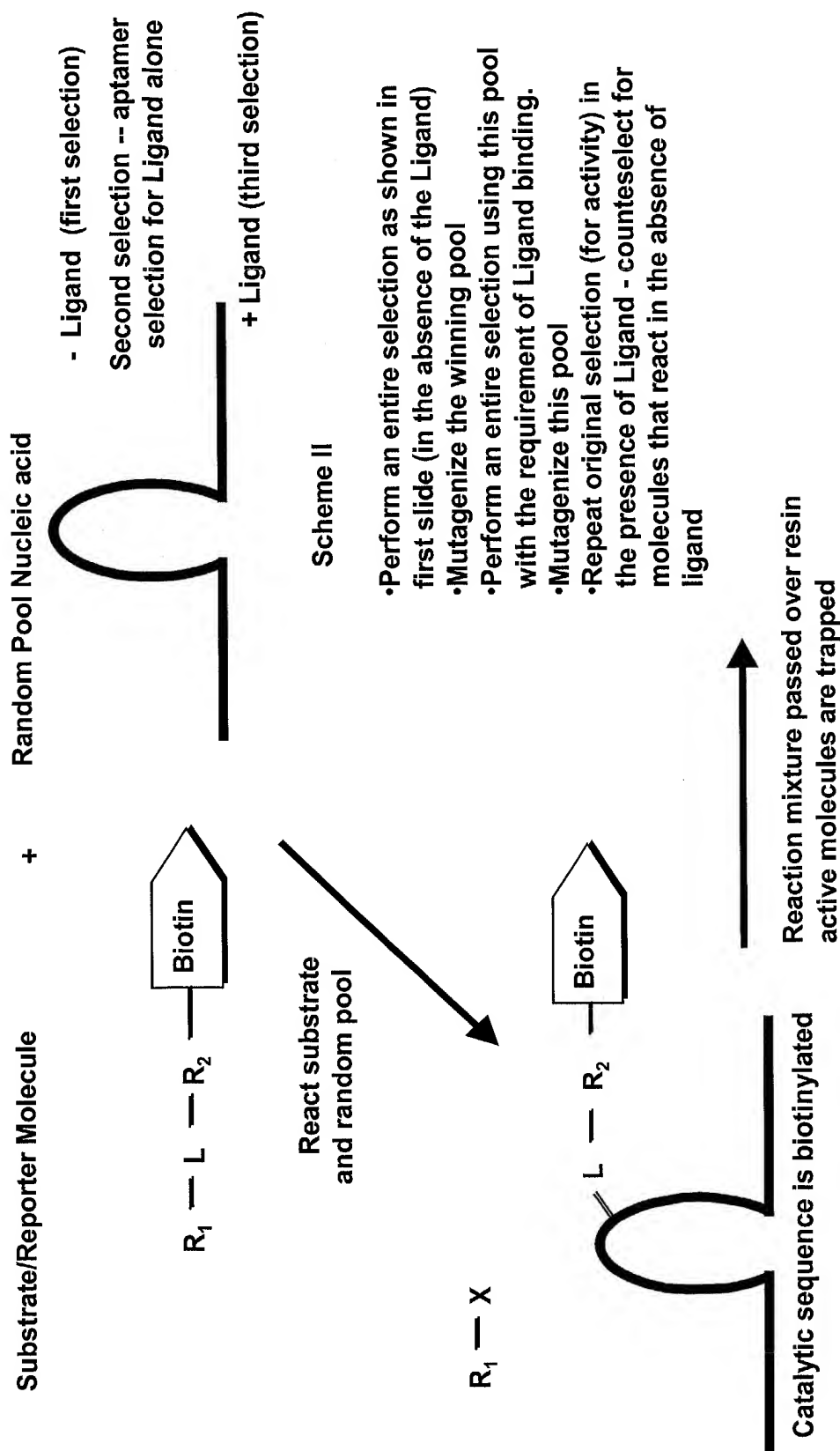
Reaction mixture passed over resin  
active molecules are trapped

## Scheme I

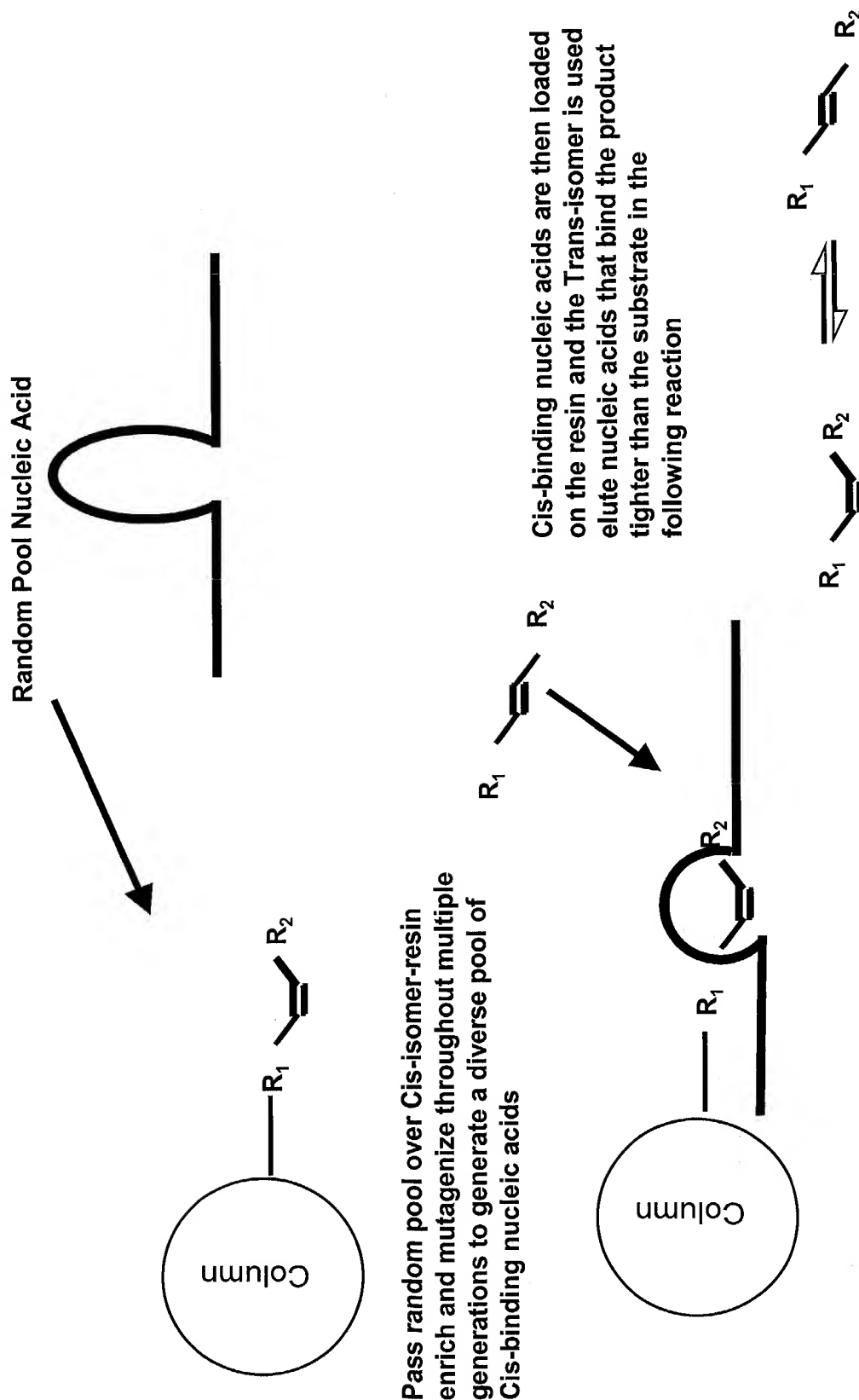
Perform this reaction (in the absence of the Ligand) and disregard the molecules that bind to the avidin resin.

Collect all RNA's that flow through the avidin resin and repeat the reaction in the presence of the Ligand. Collect and RT-PCR amplify and transcribe these molecules for subsequent rounds.

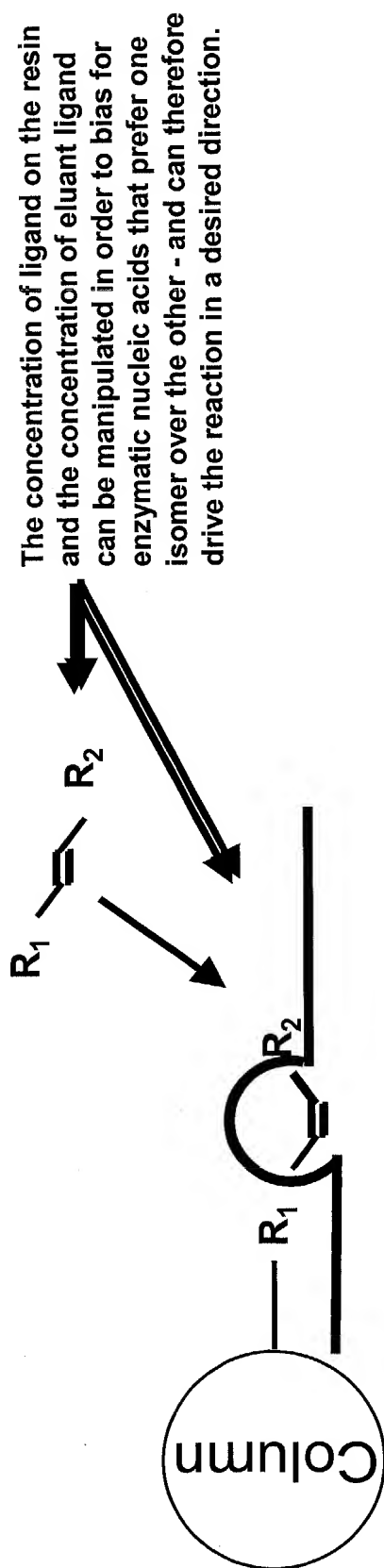
**Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules -  
Ligand dependent**



**Figure 18a: Isomerase Nucleic Acid Sensor Molecule –  
Selection Scheme**



## Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme



E.g. Selection for Cis-isomer at 100  $\mu\text{M}$  - yield  $^{\text{cis}}K_d = 100 \mu\text{M}$   
 Elute with Trans-isomer at 0.1  $\mu\text{M}$  - yield  $^{\text{trans}}K_d = 0.1 \mu\text{M}$

Isolate catalysts for the reaction below



**Figure 18c: Isomerase Nucleic Acid Sensor Molecule - Ligand dependent**

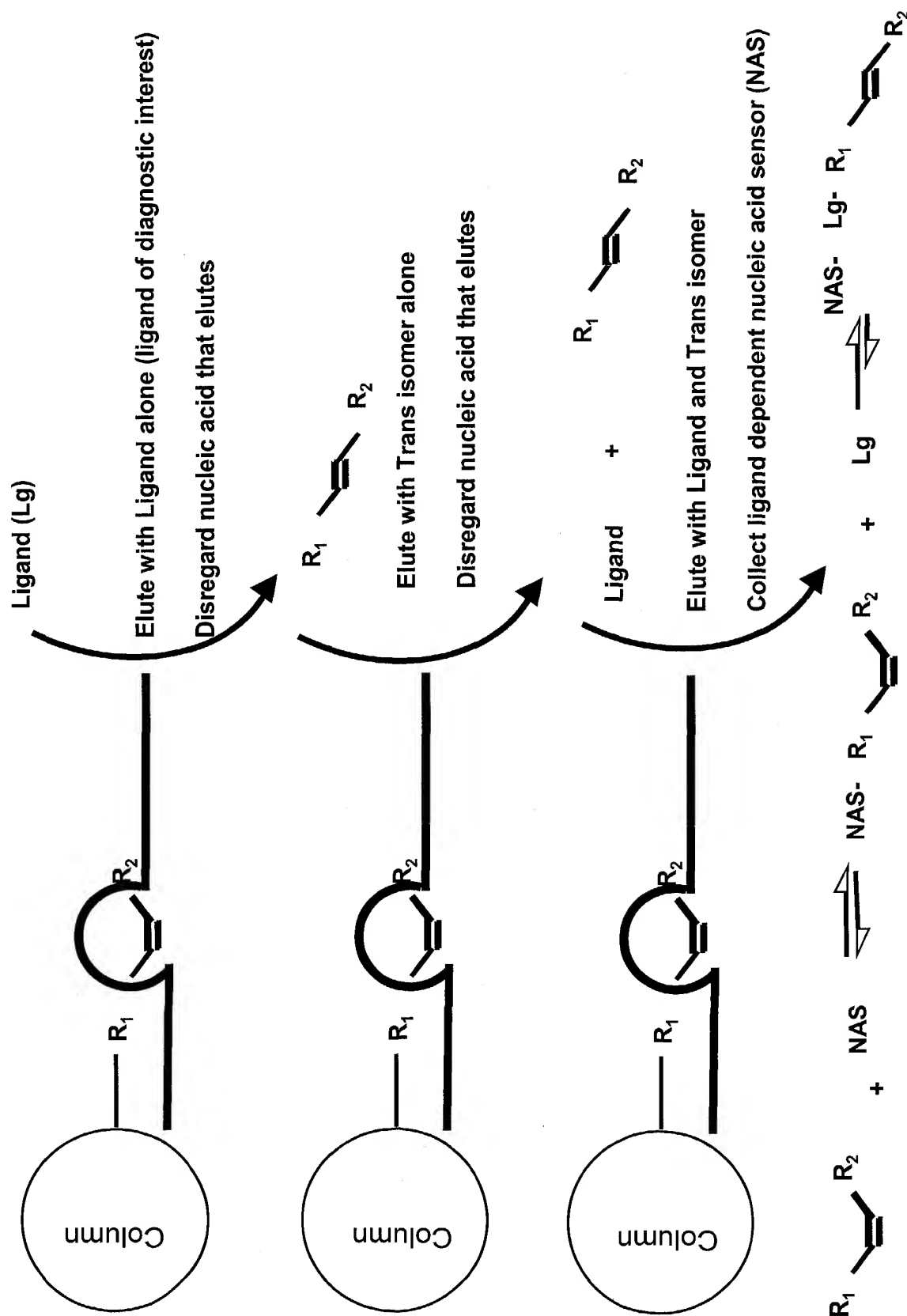
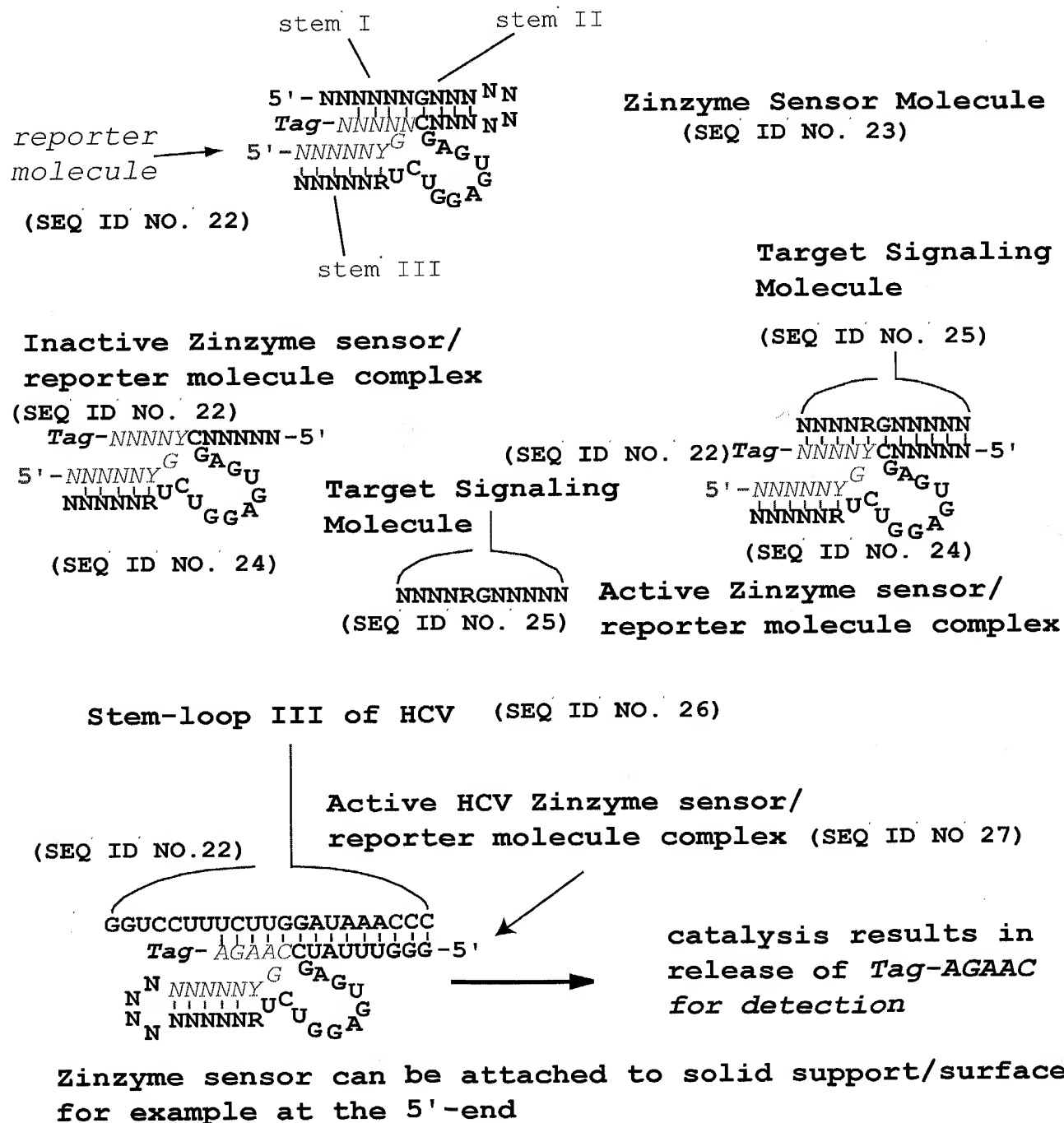
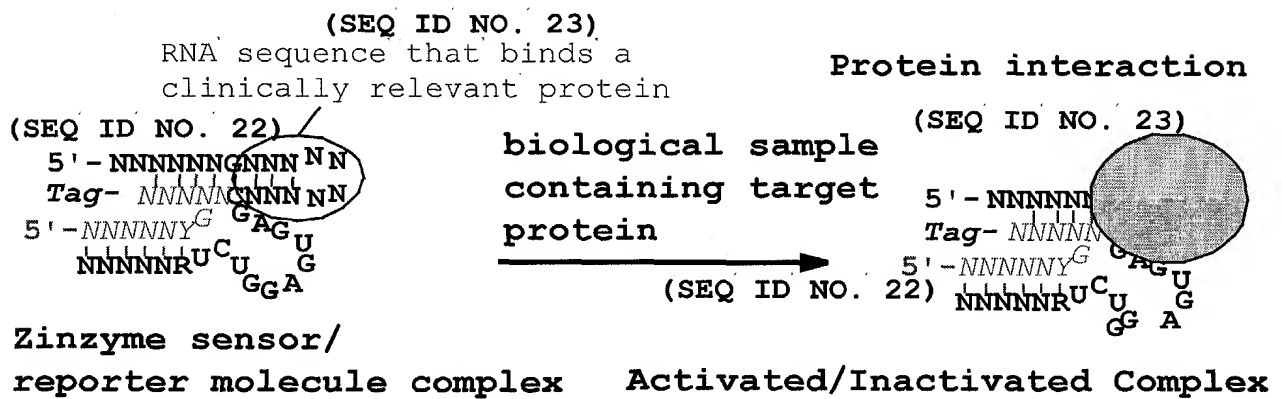




Figure 19: Zinzyme Sensor Molecule for detection of Nucleic Acid





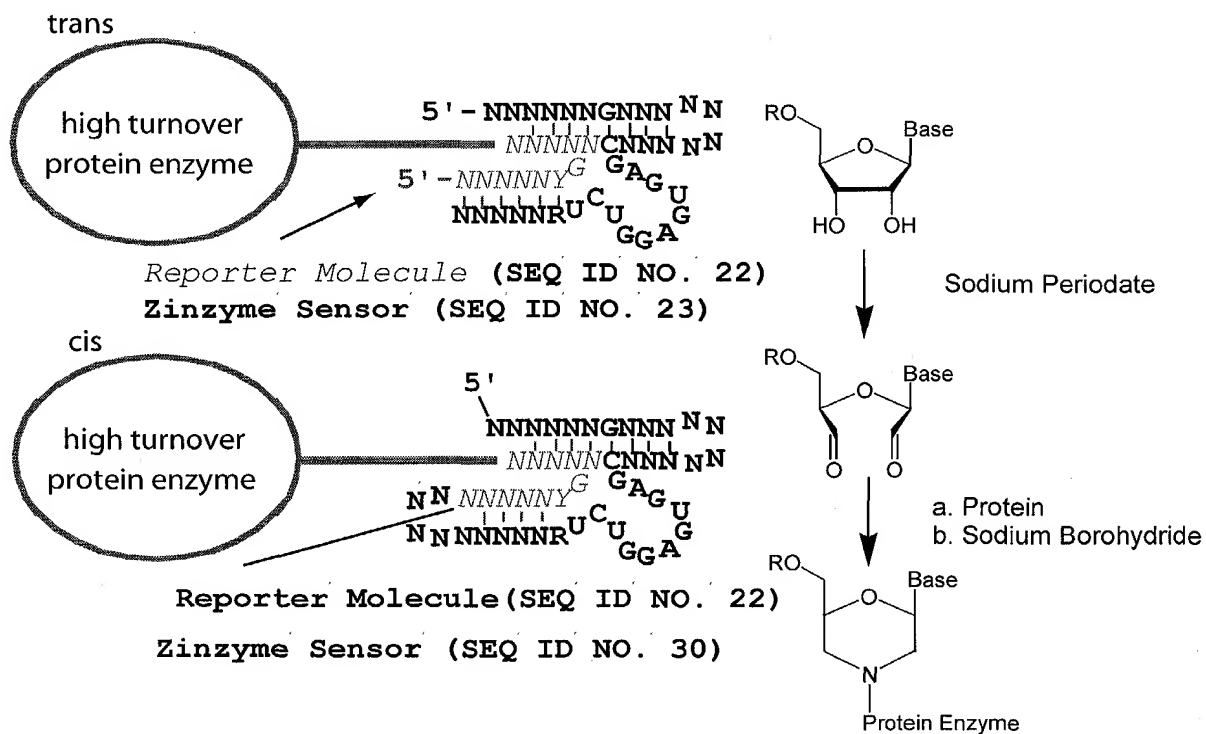
5'-NNNNNNGAGCCGCGUU  
 Tag-NNNNNCCUGGCGUG  
 5'-NNNNNYGAGUAAUG  
 NNNNNRUCUG (SEQ ID NO. 22) NNNNNRUCUGA

(SEQ ID NO. 22)  
 HCV Zinzyme sensor with  
 loop IIID of HCV  
 (directs the binding of HCV  
 core protein)  
 (SEQ ID NO 28)

5'-NNNNNNGNNNNNNNNNAGCCGCGUU  
 Tag-NNNNNCCNNNNNNNNNUGGCGUG  
 5'-NNNNNYGAGUAAUG  
 NNNNNRUCUGA

HCV Zinzyme sensor with loop III  
 of HCV connected via randomized  
 linker  
 (SEQ ID NO 29)

Figure 21: Zinzyme Sensor Molecule with protein enzyme reporter



R is oligonucleotide.

Protein can be attached via amino linker.

Alternately, R is phosphoramidite moiety for incorporation at 5'-end of oligonucleotide.

High turnover protein enzyme is, for example, Luciferase, Horseradish peroxidase, beta-galactosidase, alkaline phosphatase.

09992160-110501

## Amplification of signal via use of protein enzyme conjugate

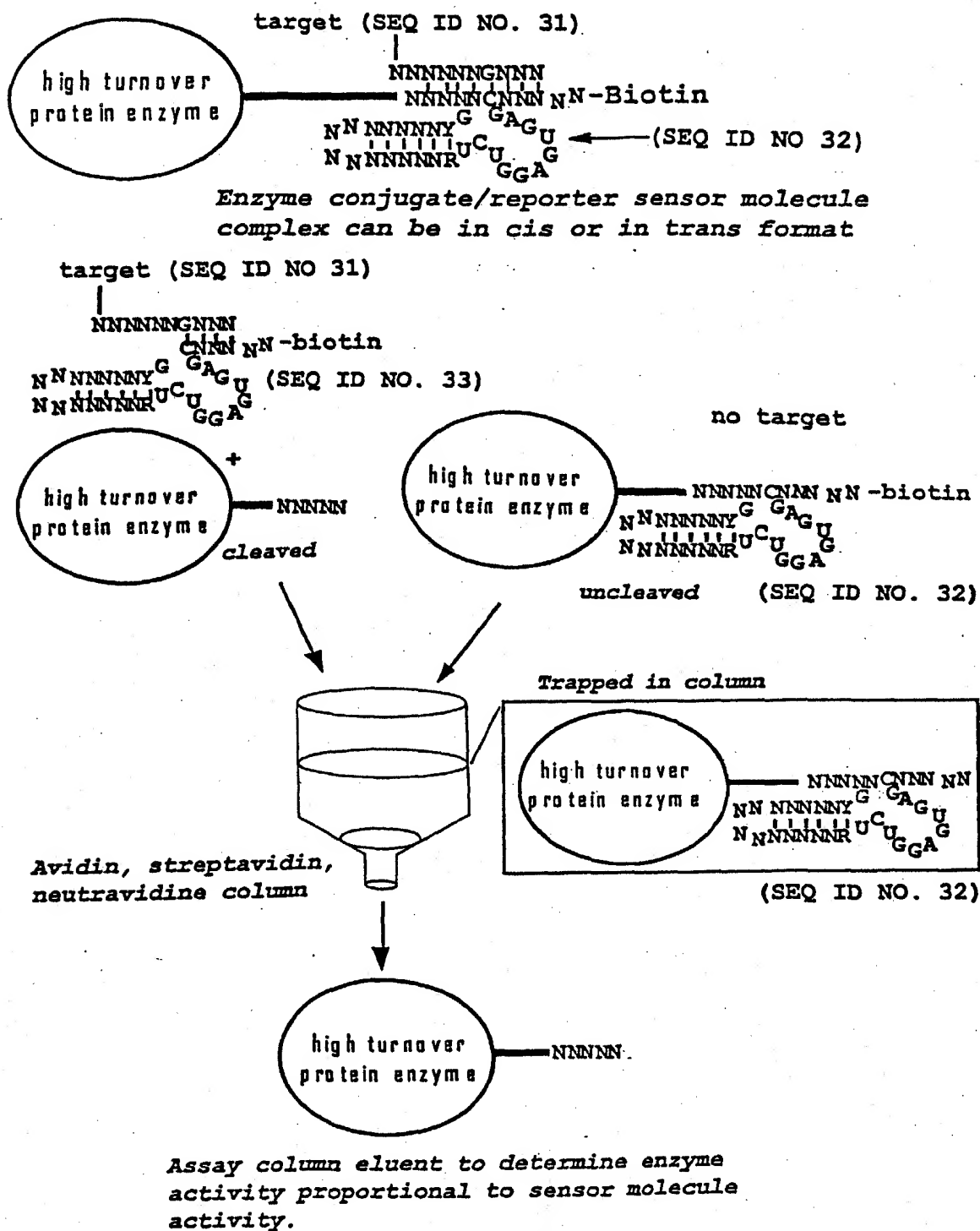
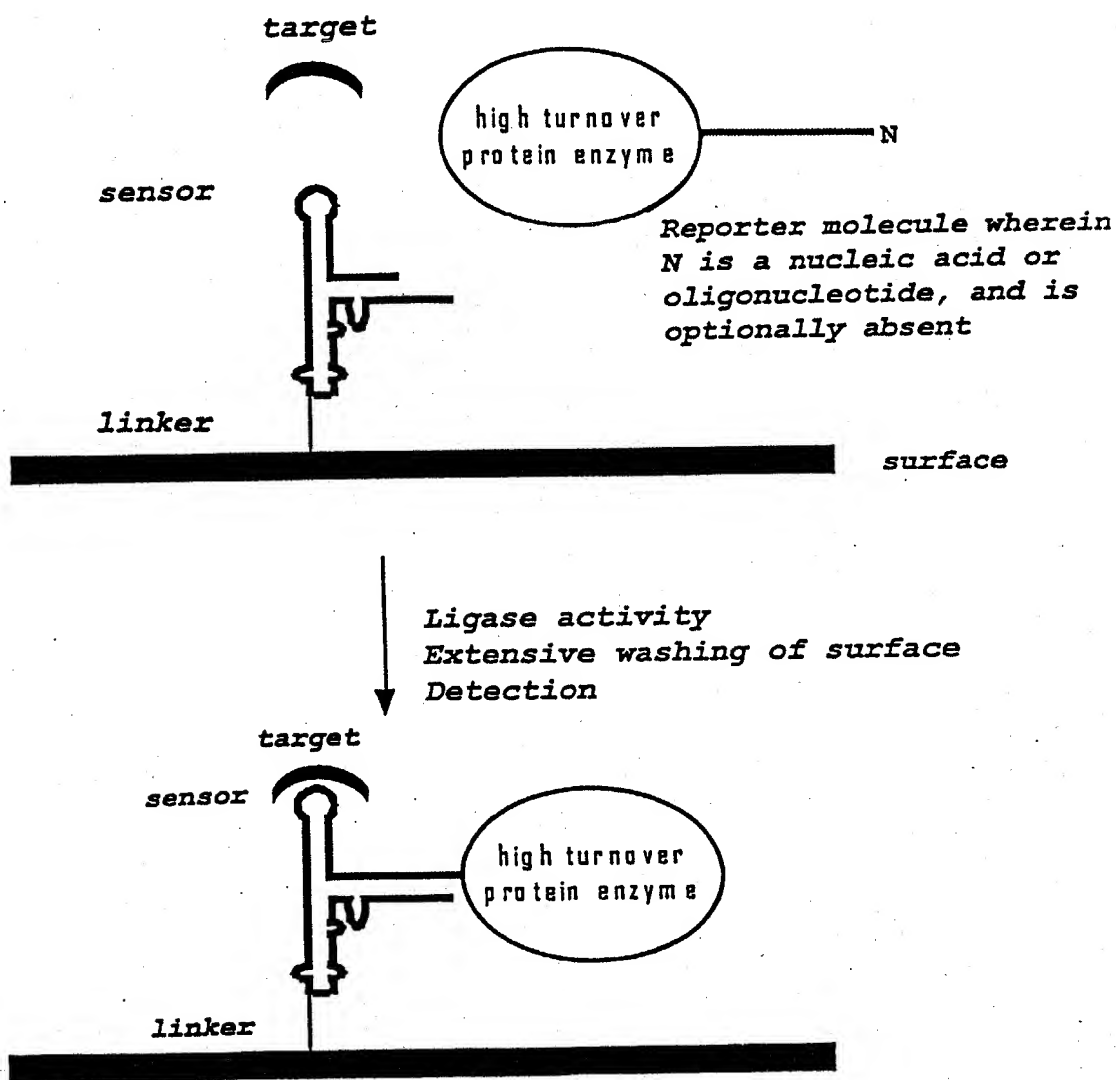


FIG. 22

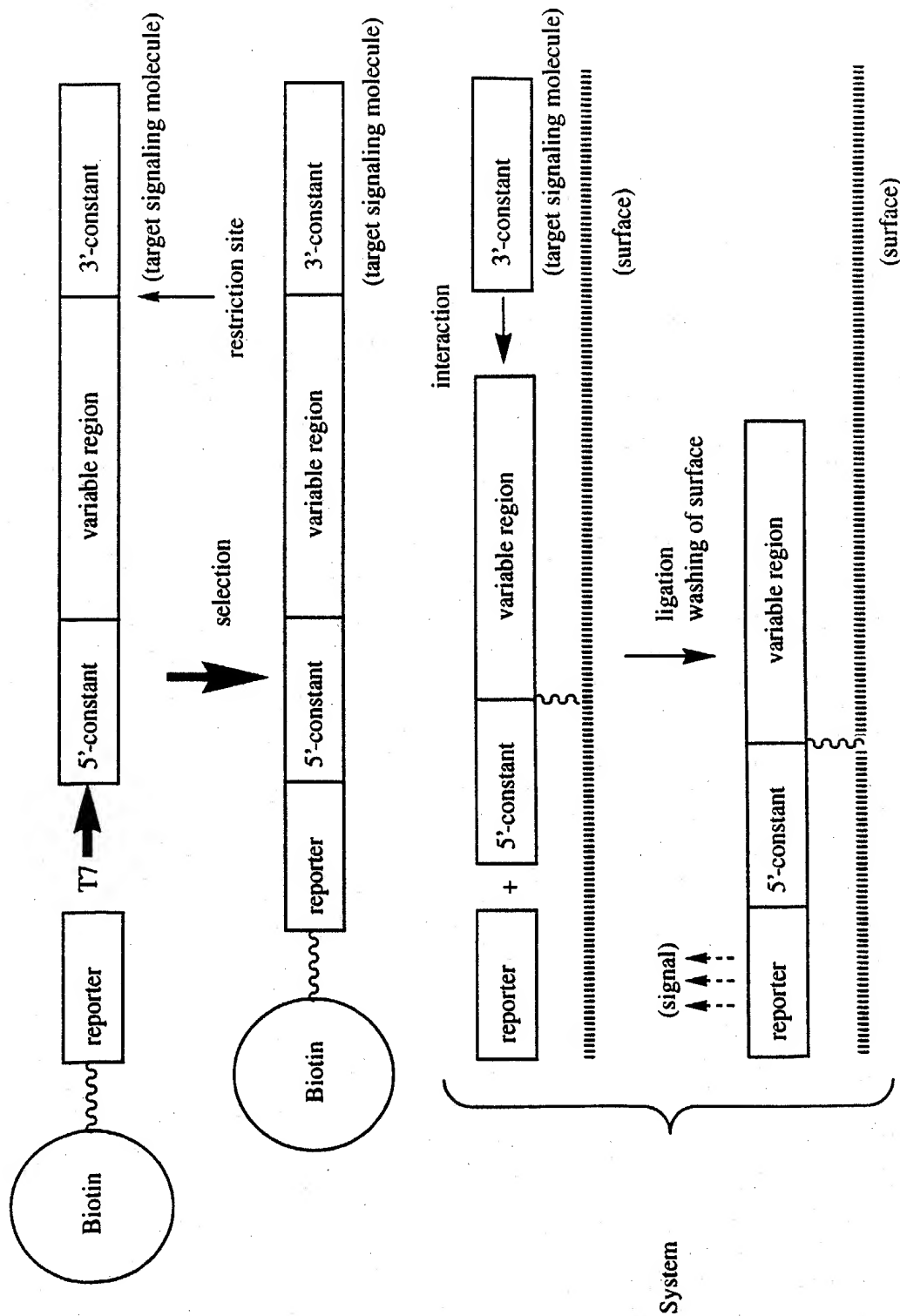
# *Ligase Sensor Molecule with enzymatic reporter*



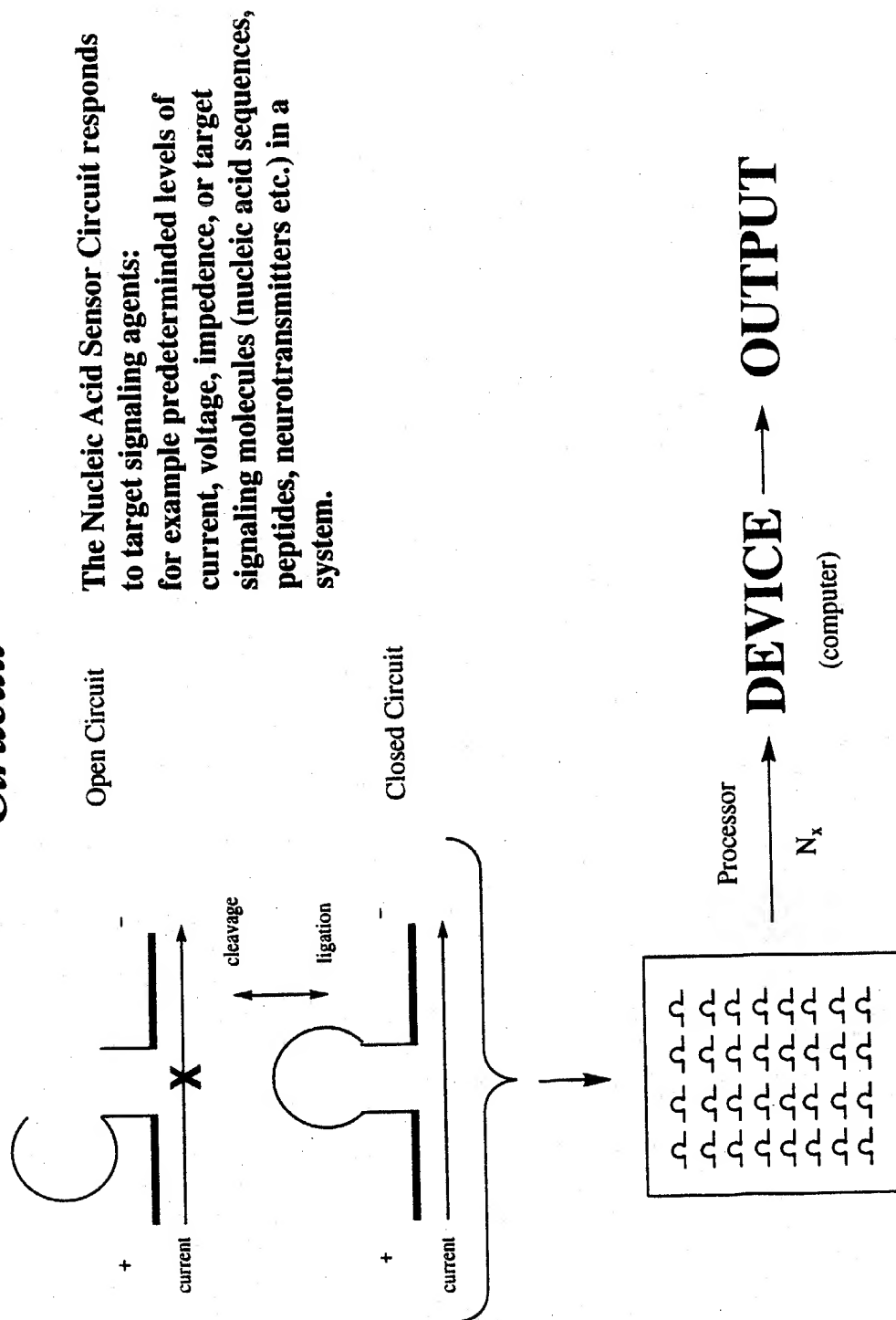
Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

FIG. 23

**Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity**



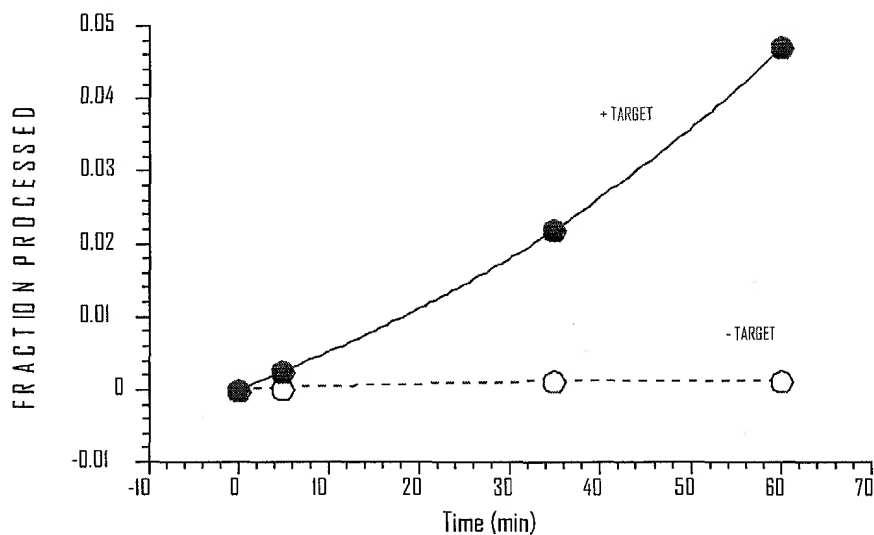
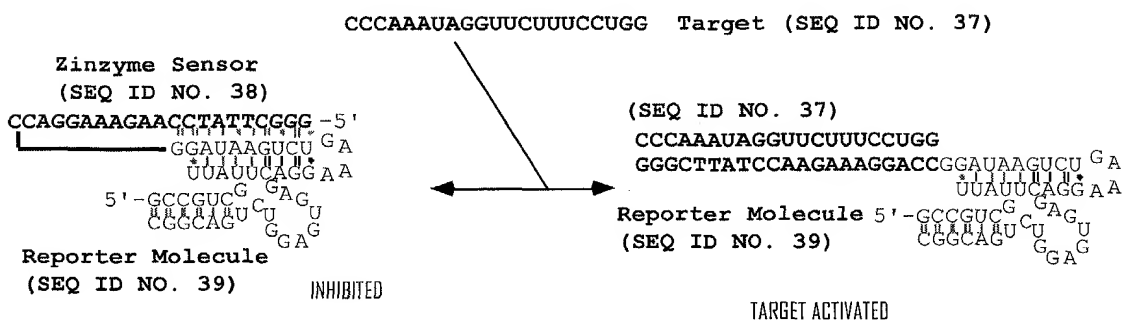
**Figure 25: Nucleic Acid Sensor Molecule-Based Electric Circuit**





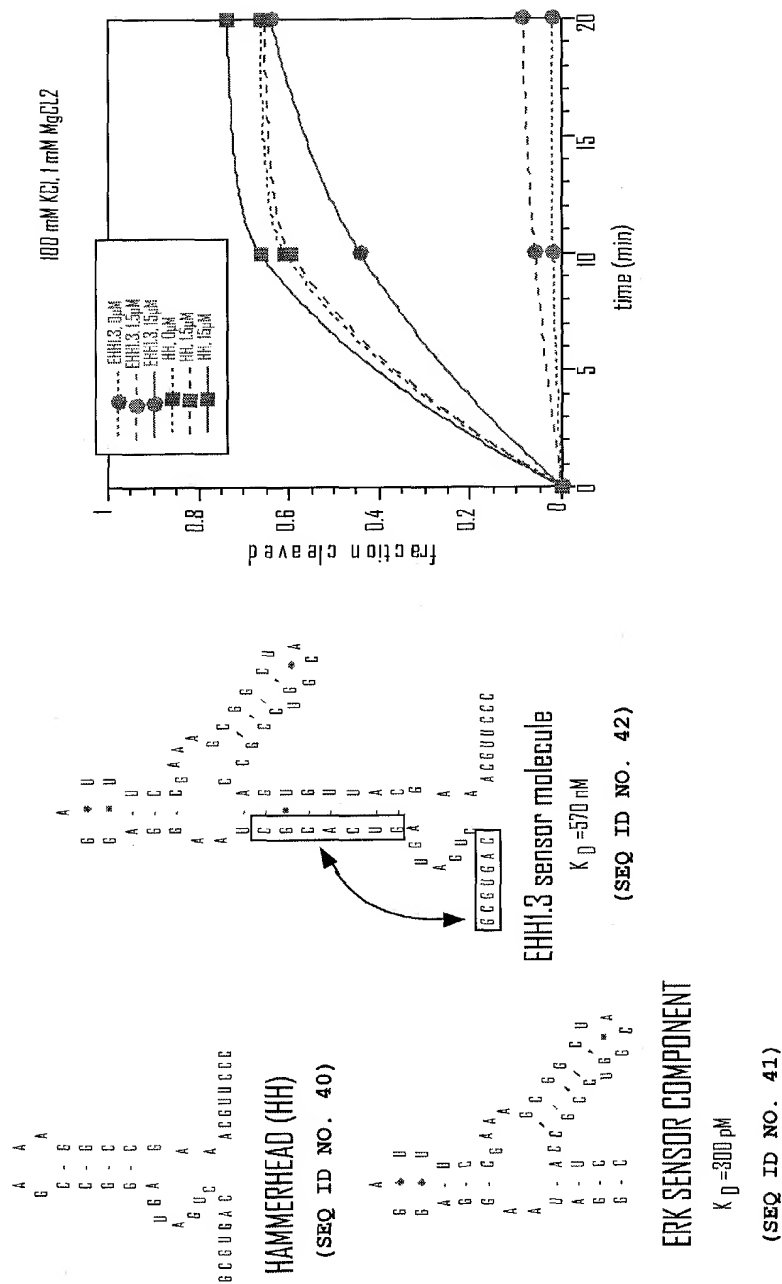


**Figure 27: Target Activation of Zinzyme Sensor Molecule**



0999150-11501

Figure 28: Erk modulated Nucleic Acid Sensor Molecule



*Figure 29: Half-Zinzyne Construct*

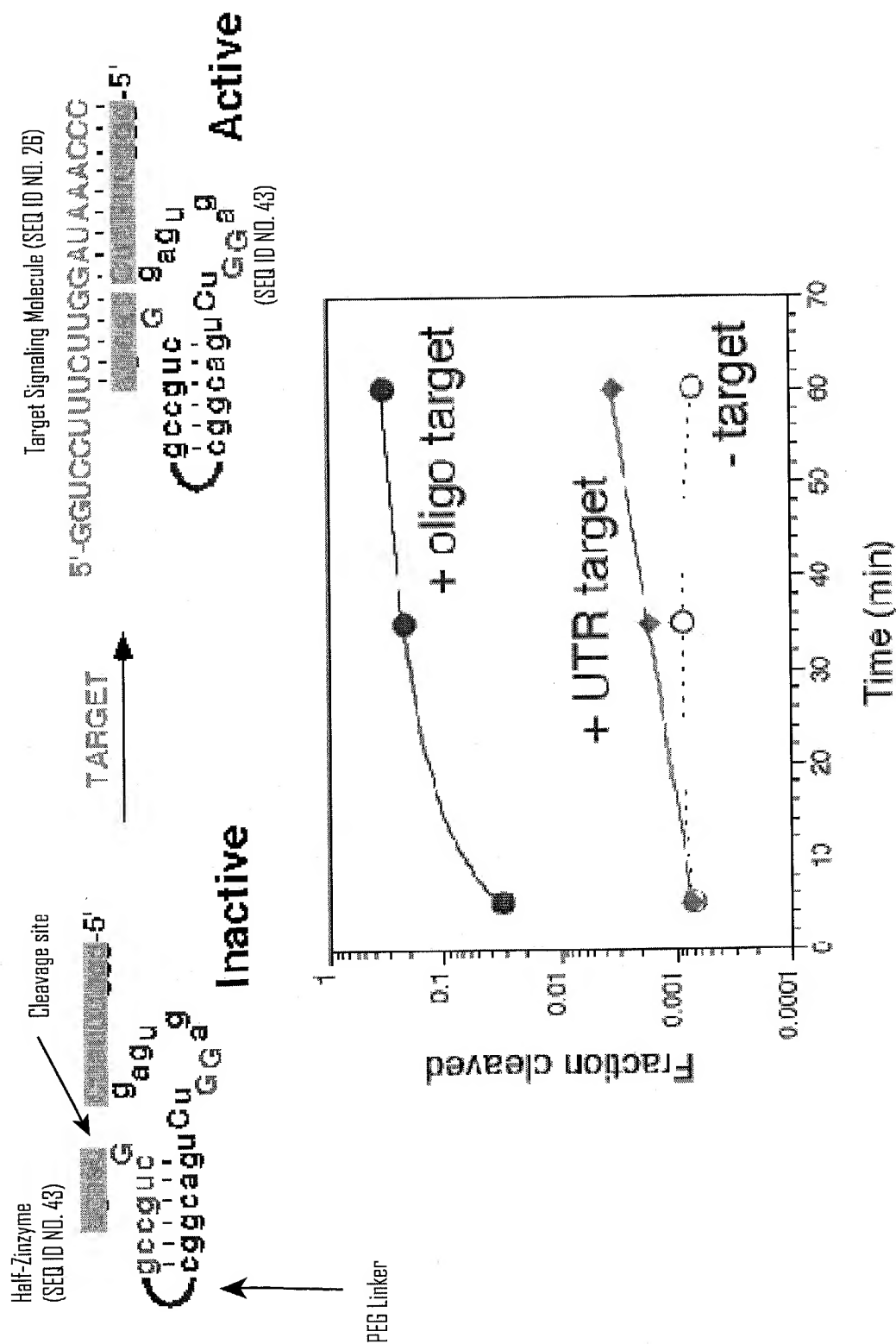


Figure 30

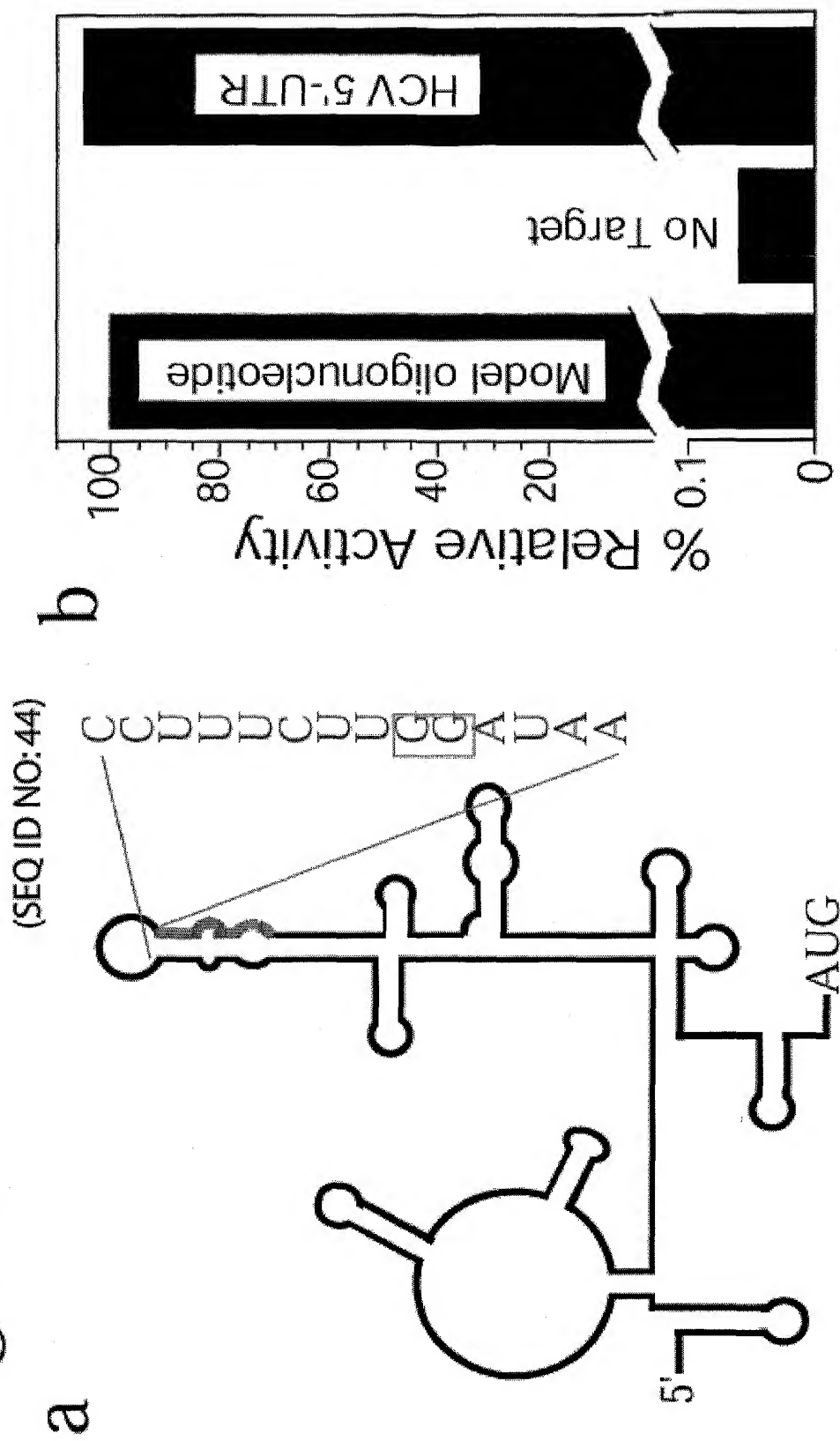


Figure 31

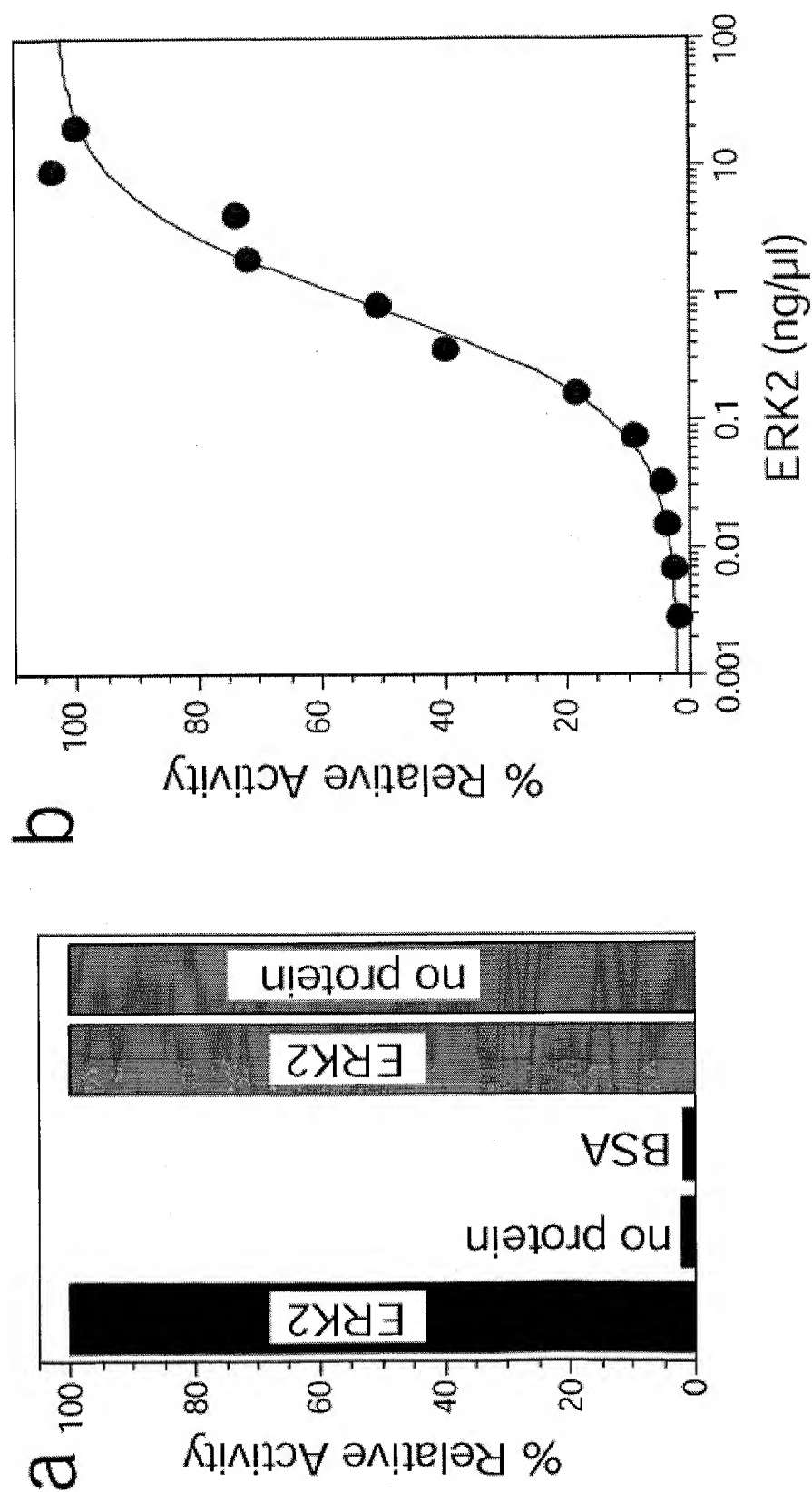
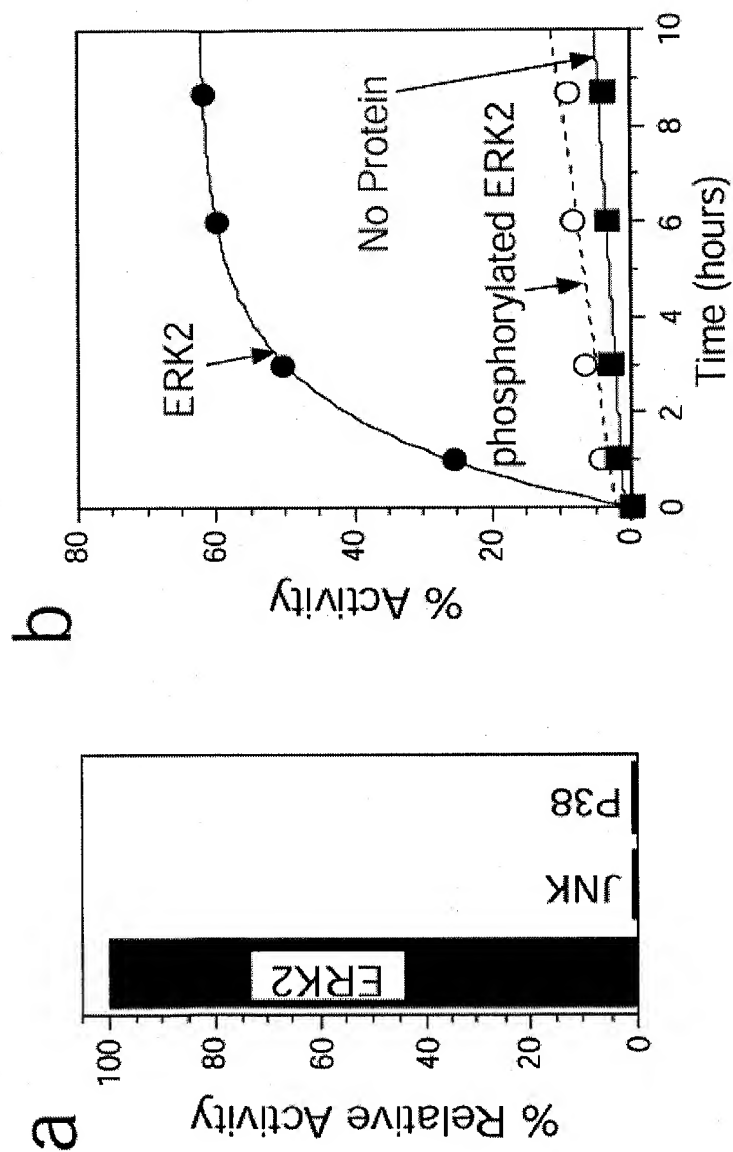


Figure 32



**Figure 33: Halfzyme Ligase**

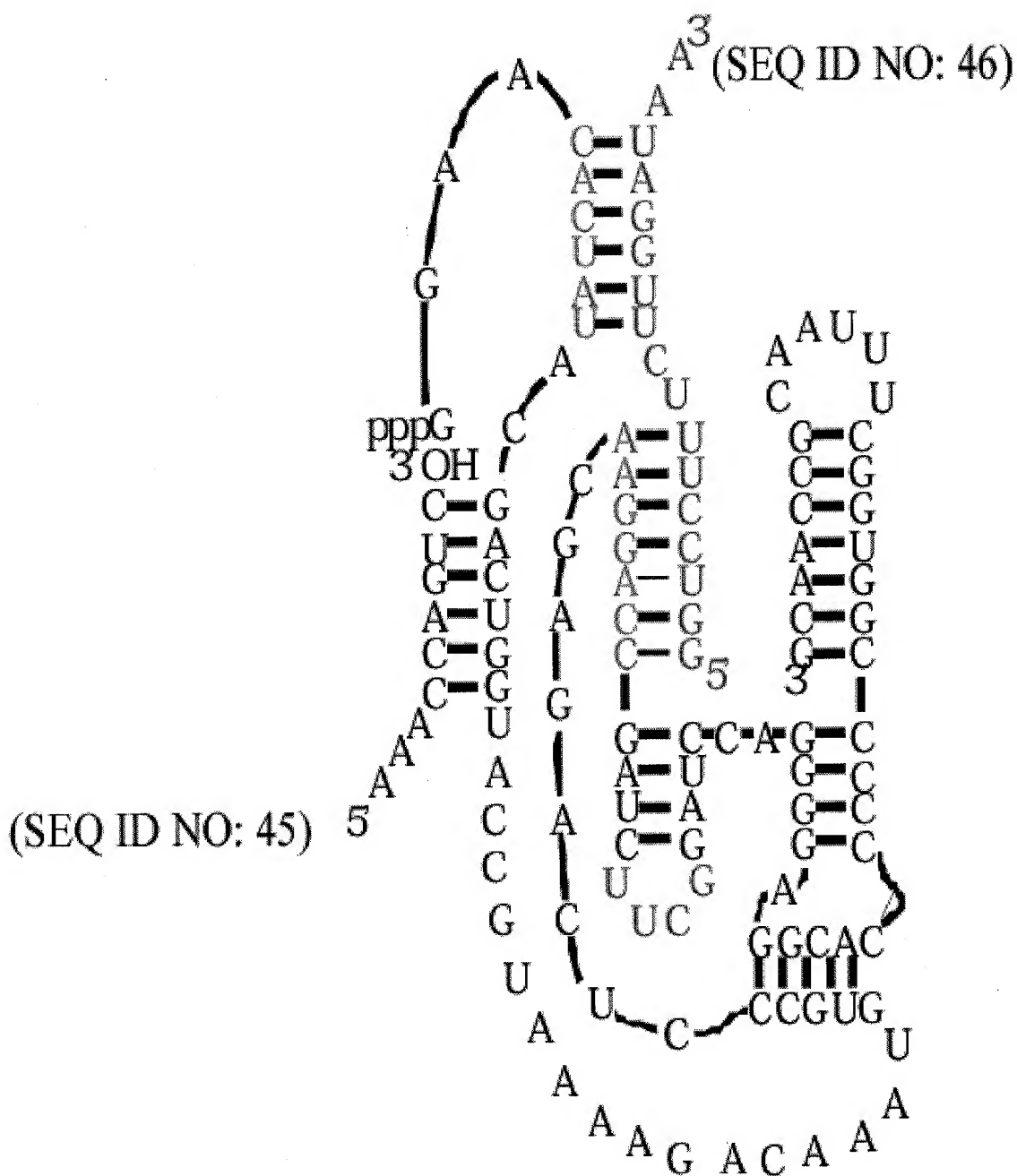
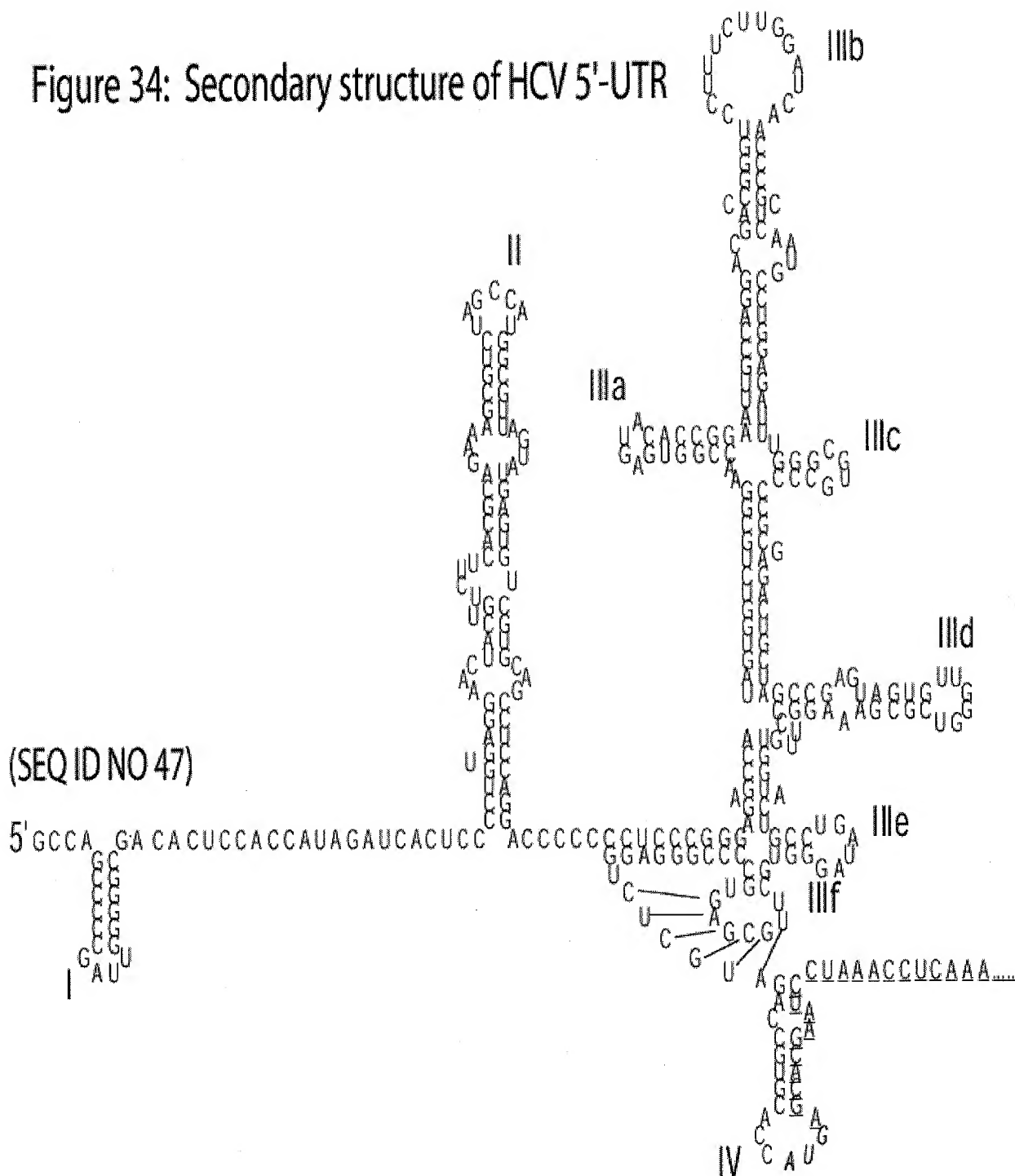


Figure 34: Secondary structure of HCV 5'-UTR





# Figure 35 Design of SNP Detection using Halfzyme-AZB7.1

SEQ ID NO:

50	51	52	53	54	55	56	57
Target HBV 1887(=AZB7-GG 3'-T C G C G - G C T G C C C C-5' (SNPT-1)	AZB7-AG 3'-T C G C A - G C T G C C C C-5' (SNPT-2)	AZB7-TG 3'-T C G C T - G C T G C C C C-5' (SNPT-3)	AZB7-CG 3'-T C G C C - G C T G C C C C-5' (SNPT-4)	AZB7-GA 3'-T C G C G - A C T G C C C C-5' (SNPT-5)	AZB7-GT 3'-T C G C G - T C T G C C C C-5' (SNPT-6)	AZB7-GC 3'-T C G C G - C C T G C C C C-5' (SNPT-7)	

58

RNA HBV 1433 3'-U C G C G - G C U G C C C C-5'

**Figure 36: Single Nucleotide Polymorphism (SNP) Detection**

